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Letters Å and Æ—see under A, letters Ø and Ö—see under O

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CLASSIFICATION AND STAGING OF MALIGNANT TUMOURS IN THE FEMALE PELVIS

Accepted by the General Assembly of FIGO in New York April 12 1970

The classification and staging of tumours in the female pelvis are considered, not only by the Cancer Committee of the International Federation of Gynecology and Obstetrics, but also by the International Union Against Cancer especially its TNM Committee, by the Cancer Unit of the WHO by the Reference Centers of the WHO for histopathological classification of ovarian tumours as well as of tumours in the uterus and the vagina, by the American Joint Committee, and finally by the International Congress of Radiology especially its committee ICPR. For several years opinions differed concerning the classification and staging of malignant tumours in the female pelvis. The Cancer Committee of FIGO has aimed at adjusting these opinions. It has been possible to reach international agreement on several main issues which are considered to be of great importance for research.

The TNM classification makes it possible to describe in detail the anatomical extent of the disease. If however used as the only method of classification it will divide series of treated cases into so many small groups that statistical evaluation of the material is not possible. The Cancer Committee of FIGO stresses the importance of staging, and experience has shown that from a statistical as well as from a prognostic point of view it is advisable to group every type of malignant tumour into four stages.

Although the UICC adheres to the TNM classification we wish to point out that there is, in principle no important difference between the classification and staging adopted by the UICC and the one adopted by FIGO as far as carcinoma of the cervix uteri, carcinoma of the corpus uteri, and carcinoma of the vagina are concerned. The FIGO clinical stage-grouping of carcinoma of the uterus and carcinoma of the vagina had already existed for many years when the proposals by the UICC were presented. Experience has shown that institutions in all countries of the world are using the FIGO clinical stage-grouping. The collaboration between the UICC and the Cancer Committee of FIGO is very important and it is of value that the so-called TNM classification of carcinoma of the uterus and carcinoma of the vagina follow the same lines as the clinical stage-grouping adopted by FIGO In this respect, however we have to remember that the stage-grouping is based exclusively on clinical examination.

General Rules for Clinical Classification and Staging of Carcinoma of the Uterus and Vagina

Every case of carcinoma of the uterus and vagina should be classified and staged prior to definitive therapy

Opinions differ as to which findings should serve as a basis for clinical classification and staging of carcinoma of the uterus and vagina. In order to obtain a correct and uniform classification and staging the Cancer Committee considers it important that exclusively such examinations should be used which can be carried out at any hospital by the physicians and surgeons. The following examinations fulfill this requirement: Palpation, inspection, colposcopy hysteroscopy fractional curettage, roentgen examination of the lungs and skeleton, and finally intravenous urography Some clinicians prefer to supplement their examination with lymphography arteriography venography hysterography laparoscopy etc. The Cancer Committee does not recommend these last mentioned examination as a basis for staging. They may sometimes give information of importance but it is evident that none of them is

used as a routine. Furthermore, opinions differ among roentgenologists about the interpretation of findings observed by for instance, lymphography venography or hysterography

Carcinoma of the Cervix

Cases should be classified as carcinoma of the cervix if the primary growth is in the cervix. All histological types must be included.

The clinical staging of carcinoma of the cervix is based on palpation inspection colposcopy endocervical curettage roentgen examination of lungs and skeleton and urography

A conisation or amputation of the cervix should be regarded as a clinical examination and an invasive carcinoma of the cervix diagnosed in this way should be reported as invasive carcinoma.

In the past, cases of invasive carcinoma which were diagnosed only at the histological examination of the removed uterus should be excluded from therapeutic statistics on invasive carcinoma. As the number of such cases is increasing, the Cancer Committee has seriously reconsidered this problem, and the Committee recommends that cases in which a microscopic focus is found in the removed uterus should be included among the Stage I cases, but be reported as Stage Ia—post surgical"

The presence of a hydronephrosis or non functioning kidney due to stenosis of the ureter by the cancer permits the allotment of a case to Stage III even if according to the other findings the case should be allotted to an earlier stage

Sub-grouping of Stage I

It is well known that opinions differ among pathologists concerning the criteria for early invasion of abnormal epithelium The introduction of colposcopy and cytology in the clinical examination has increased the number of early carcinoma. The number of such cases at an institution will partly depend on the pathologist and his criteria for considering a case as invasive. Cases of early invasive carcinoma sometimes are treated in a different way than are cases of obviously invasive carcinoma. Therefore, the Cancer Committee some years ago recommended a sub-division of the Stage I cases into Stage Ia—cases which cannot be diagnosed by clinical examination, and Stage Ib—all other cases of Stage I

To Stage Ia should be allotted cases of pre clinical cancer Consequently this group will include cases of early stromal invasion and some cases of occult invasive cancer Cases of occult cancer may be found, for instance in a cone or in the specimen from a large wedge biopsy performed in cases of carcinoma in situ or dysplasia

In order to establish as explicit and uniform rules as possible the Cancer Committee recommends a sub-division of the Stage Ia cases into (i) early stromal invasion, and (ii) occult cancer We emphasize that there can never be a sharp line between Stage Ia and Stage Ib. We regard it as statistically important, and from a therapeutic point of view perhaps of value, to have information about the proportion of early Stage I cases.

To Stage Ia should also be allotted, as already mentioned above those cases in which a microscopic focus is found in the removed uterus. They should be reported as Stage Ia—postsurgical

Sub-grouping of Stage II

Some years ago the Cancer Committee recommended a sub-grouping of the Stage II cases into IIa—no parametrial involvement, and IIb—obvious parametrial involvement. This sub-grouping has been widely used but has not been officially approved by the General Assembly until at its meeting in April 1970 in New York. The clinical staging is of importance not only to compare therapeutic results, but also to give information of value when planning therapy Irrespective of method of therapy applied the treatment is different if the parametrium is obviously involved or not, and therefore the Cancer Committee recommends the following subgrouping:

Stage IIa The carcinoma involves the vagina but not the lower third. No obvious extension to the parametrium.

Stage IIb The carcinoma involves the vagina but not the lower third. Obvious extension to the parametrium, but not on to the pelvic wall

Sub-grouping of Stage III

For the same reasons as above the Cancer Committee recommends a subgrouping of the Stage III cases along the same lines as for Stage II i.e

Stage IIIa The carcinoma involves the lower third of the vagina but has not extended on to the pelvic wall

Stage IIIb The carcinoma has extended on to the pelvic wall.

Stage IV

The Cancer Committee recommends that a bulloos oedema as such should not permit allotment of a case to Stage IV Ridges and furrows into the bladder wall should be interpreted as signs of submucous involvement of the bladder if they remain fixed to the growth at palpocopy i.e. examination from the rectum during cystoscopy A cytological finding of malignant cells in washings from the urinary bladder requires further examination and a biopsy from the wall of the bladder

Definitions of the different clinical stages in carcinoma of the cervix uteri. (To be used from January 1 1971)

Pre-invasive carcinoma

Stage 0 Carcinoma in situ, intraepithelial carcinoma. Cases of Stage 0 should not be included in any therapeutic statistics.

Invasive carcinoma

Stage I Carcinoma strictly confined to the cervix (extension to the corpus should be disregarded).

Ia. The cancer cannot be diagnosed by clinical examination (i) early stromal invasion, and (b) occult cancer

Ib All other cases of Stage I

Stage II The carcinoma extends beyond the cervix but has not extended on to the pelvic wall. The carcinoma involves the vagina, but not the lower third

IIa No obvious parametrial involvement.

Stage III The carcinoma has extended on to the pelvic wall. On rectal examination there is no cancer free space between the tumour and the pelvic wall The tumour involves the lower third of the vagina

IIIa No extension on to the pelvic wall.

IIIb Extension on to the pelvic wall.

Stage IV The carcinoma has extended beyond the true pelvis or has involved the mucosa of the bladder or rectum A bullous oedema as such does not permit allotment of a case to Stage IV

Carcinoma of the Corpus

A case should be classified as carcinoma of the corpus uteri when the primary site of the growth is in the corpus. Cases of mixed mesenchymal tumours and so-called carcinosarcoma should be excluded.

Stage 0 Carcinoma in situ. Histological findings suspicious of malignancy Cases of Stage 0 should not be included in any therapeutic statistics.

Stage I The carcinoma is confined to the corpus. The great majority of cases of carcinoma of the corpus will be allotted to Stage I. The factors which decide the prognosis in carcinoma of the corpus Stage I are 1) the age and the general condition of the patient, 2) the size of the uterine cavity and 3) the histological pattern.

Several years ago the Annual Report on the Results of Treatment in Carcinoma of the Uterus recommended a sub-division of the Stage I cases with regard to operability Patients of old age and patients suffering from extra-genital disease were considered as poor operative risks. Experience has obviously shown that no absolute criteria can be given for poor operative risk Such a judgement depends on the ability and skill of the surgeon.

The Cancer Committee of FIGO considers a sub-division with regard to operability of limited value. We will not deny however that especially in presentations of therapeutic results in carcinoma of the corpus it is of value to know the number of patients suffering from serious extra-genital disease, as for instance nephrocardiovascular disabilities, and the number of patients of 70 or 80 years of age, respectively

Studies of large series of endometrial carcinoma limited to the corpus have shown that the prognosis to some extent is related to the size of the uterus. However enlargement of the uterus may be caused by fibroids, adenomyosis, etc. Therefore, *the size* of the uterus cannot serve as a basis for sub-grouping Stage I cases. The length *of the uterine cavity* however measured with a sound from the external os, may give information of value for therapy The Cancer Committee recommends a sub-division of the Stage I cases *with regard to the length of the sound.* Cases with sound of more than 8 cm to Stage Ib

The histo-pathology as such should not serve as a basis for clinical stage-grouping. Experience however has demonstrated that highly differentiated adenocarcinoma, frequently with papillary structures, as well as differentiated carcinoma

with partly solid areas have a tendency to grow exophytically in the uterine cavity while predominantly solid and entirely undifferentiated anaplastic tumours frequently tend to deeply invade the myometrium at an early stage of development. Thus, these last mentioned tumours are more malignant and experience has shown that they have a worse prognosis than the differentiated ones. A sub-division of the Stage I cases with regard to the histological structures will facilitate the interpretation of a series of carcinoma of the corpus Stage I. Therefore, the Cancer Committee recommends *a sub-division of the Stage I cases in*.

G 1 highly differentiated adenomatous carcinomas

G 2 differentiated adenomatous carcinomas with partly solid areas

G 3 predominantly solid or entirely undifferentiated carcinomas.

Stage II The carcinoma has involved the corpus and the cervix. As far as prognosis and therapy are concerned it is important to know whether the cancer has extended to the cervix. The extension of the carcinoma to the endocervix is confirmed by fractional curettage or hysteroscopy The scraping of the cervix should be the first step of the curettage and the specimens from the cervix should be examined separately Occasionally it may be difficult to decide whether the endocervix is involved by the cancer or not. In such cases simultaneous presence of normal cervical glands and cancer in the same piece will give the final diagnosis. In questionable cases the histological examination of the curettage should decide whether the origin of the cancer is in the corpus or in the cervix. If a clear decision cannot be made an adenocarcinoma should be allotted to carcinoma of the corpus and an epidermal carcinoma to carcinoma of the cervix.

Stage III and Stage IV Extension of the carcinoma outside the uterus should refer a case to Stage III or Stage IV

The presence of metastases in the vagina permits, as such, to allot a case to Stage III.

Every gynaecologist and pathologist knows that there are cases in which it is clinically as well as histologically impossible to decide whether the cancer primarily is a cancer of the corpus uteri or a cancer of the ovary Previously such cases were diagnosed as carcinoma uteri et ovarii but this is not adequate. As a rule, it is possible from the history of the patient and from the clinical examination to decide which tumour is likely to be the primary one. In rare cases this may however be impossible. Such rare cases should be included in the statistics on carcinoma of the corpus as well as in the statistics on ovarian cancer They should be reported separately

Definitions of the different clinical stages of carcinoma of the corpus uteri (To be used from January 1 1971)

Stage 0 Carcinoma in situ. Histological findings suspicious of malignancy

Cases of Stage 0 should not be included in any therapeutic statistics.

Stage I The carcinoma is confined to the corpus.

Ia. The length of the uterine cavity is 8 cm or less.

Ib The length of the uterine cavity is more than 8 cm.

The Stage I cases should be sub-grouped with regard to the histological type of the adenocarcinoma as follows.

G 1 highly differentiated adenomatous carcinomas,

G 2. differentiated adenomatous carcinomas with partly solid areas

G 3 predominantly solid or entirely undifferentiated carcinomas.

Stage II The carcinoma has involved the corpus and the cervix.

Stage III The carcinoma has extended outside the uterus but not outside the true pelvis.

Stage IV The carcinoma has extended outside the true pelvis or has obviously involved the mucosa of the bladder or rectum. A bullous oedema as such does not permit allotment of a case to Stage IV

Carcinoma of the Vagina

Cases should be classified as carcinoma of the vagina when the primary site of the growth is in the vagina. Tumours presenting in the vagina as secondary growths from either genital or extragenital sites should be excluded from registration.

A growth that has extended to the portio and reached the area of the external os should always be allotted to carcinoma of the cervix

A growth that has extended to the vulva should be classified as a carcinoma of the vulva.

A growth that is limited to the urethra should be classified as a carcinoma of the urethra.

Definitions of the different clinical stages i car cinoma of the vagina. (To be used from January 1 1962)

Pre-invasive arcinoma

Stage 0 Carcinoma in situ, intraepithelial car cinoma.

I vasive carcinoma

Stage I The carcinoma is limited to the vaginal wall.

Stage II The carcinoma has involved the sub-vaginal tissue but has not extended on to the pelvic wall.

Stage III The carcinoma has extended on to the pelvic wall.

Stage IV The carcinoma has extended beyond the true pelvis or has involved the mucosa of the bladder or rectum. A bullous oedema as such does not permit allotment of case to Stage IV

Carcinoma of the Vulva

Cases should be classified as carcinoma of the ul a when the primary site of the growth is in the ul a. Tumours present in the vulva as second ary growths from either a genital or extra-genital te should be excluded from registration as should cases of malignant melanoma (see also carcinoma of the vagina)

Many attempts ha e been made to classify and stage carcinoma of the vulva (Sanon 193., Taussig 1940 Berven 1941 Cosbie 1951), but none of the proposals has been internationally accepted.

Carcinoma of the vulva tends to give metastases prima il) to the inguinal nodes Only exceptionally the primary site of metastases from a carcinoma of the ul s in the deep pel ic lymph nodes. Therefore, the TNM classification is well suited for this tumour In 1967 the TNM Committee of the UICC proposed classification for car cinoma of the vulva. The Cancer Committee of FIGO has expressed some criticism against the UICC proposal.

The Cancer Committee of FIGO wishes to ex press it sincere thanks to M Stanley W y New castle Dr Howard Ulfelder Boston, Dr Malcolm Stening, Sydney and Dr Folke Edsmyr Stockholm, for their detailed investigations of the series of carcinoma of the vulva It is important to point out that, in principle, the four colleagues came to the same conclusions. At the congress in Sydney the Cancer Committee discussed some minor differences in the recommendations presented by the above mentioned colleagues. At the Radium hemmet a series of 721 cases of primary invasive epidermoid cancer of the vulva treated by primary surgery has been investigated by Edsmyr and Kottmeier In April 1970 the General Assembly of FIGO approved on a TNM classification and a stage-grouping of carcinoma of the vulva proposed by the Cancer Committee. The FIGO classi fication differs to some extent from the UICC proposal.

TNM classification and clinical staging of car cinoma of the vulva. (Adopted in 1970, to be used from January 1 1971)

T Primary tumour

T 1 Tumour confined to the vulva—2 cm or less in larger diameter

T .. Tumour confined to the vulva—more than cm in diameter

T 3 Tumour of any size with adjacent spread to the urethra and/or vagina and/or perineum and/or the anus.

T 4 Tumour of any size infiltrating the bladder mucosa and/or the rectum mucosa or both, including the upper part of the urethra mucosa and/or fixed to the bone.

N Regional lymph nodes

N 0: No nodes palpable.

N 1 Nodes palpable in either groin, not enlarged, mobile (not clinically suspicious of neoplasm).

N 2. Nodes palpable in either one or both groins, enlarged, firm and mobile (clinically suspicious of neoplasm).

N 3 Fixed or ulcerated nodes.

M Distant metastases

M 0 No clinical metastases.

M 1a. Palpable deep pelvic lymph nodes

M 1b. Other distant metastases.

Clinical Stage Groups

Stage I	*Stage II*
T 1 N 0 M 0	T N 0 M 0
T 1 N 1 M 0	T N 1 M 0

with partly solid areas have a tendency to grow exophytically in the uterine cavity while predominantly solid and entirely undifferentiated anaplastic tumours frequently tend to deeply invade the myometrium at an early stage of development. Thus, these last mentioned tumours are more malignant and experience has shown that they have a worse prognosis than the differentiated ones. A sub-division of the Stage I cases with regard to the histological structures will facilitate the interpretation of a series of carcinoma of the corpus Stage I Therefore, the Cancer Committee recommends *a sub-division of the Stage I cases in*

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G 3 predominantly solid or entirely undifferentiated carcinomas.

Stage II The carcinoma has involved the corpus and the cervix. As far as prognosis and therapy are concerned it is important to know whether the cancer has extended to the cervix. The extension of the carcinoma to the endocervix is confirmed by fractional curettage or hysteroscopy The scraping of the cervix should be the first step of the curettage and the specimens from the cervix should be examined separately Occasionally it may be difficult to decide whether the endocervix is involved by the cancer or not. In such cases simultaneous presence of normal cervical glands and cancer in the same piece will give the final diagnosis. In questionable cases the histological examination of the curettage should decide whether the origin of the cancer is in the corpus or in the cervix. If a clear decision cannot be made an adenocarcinoma should be allotted to carcinoma of the corpus and an epidermal carcinoma to carcinoma of the cervix.

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The presence of metastases in the vagina permits, as such, to allot a case to Stage III

Every gynaecologist and pathologist knows that there are cases in which it is clinically as well as histologically impossible to decide whether the cancer primarily is a cancer of the corpus uteri or a cancer of the ovary Previously such cases were diagnosed as carcinoma uteri et ovarii but this is not adequate. As a rule, it is possible from the history of the patient and from the clinical examination to decide which tumour is likely to be the primary one. In rare cases this may however be impossible. Such rare cases should be included in the statistics on carcinoma of the corpus as well as in the statistics on ovarian cancer They should be reported separately

Definitions of the different clinical stages of carcinoma of the corpus uteri (To be used from January 1 1971)

Stage 0 Carcinoma in situ. Histological findings suspicious of malignancy

Cases of Stage 0 should not be included in any therapeutic statistics.

Stage I The carcinoma is confined to the corpus.

Ia. The length of the uterine cavity is 8 cm or less.

Ib The length of the uterine cavity is more than 8 cm.

The Stage I cases should be sub-grouped with regard to the histological type of the adenocarcinoma as follows.

G 1 highly differentiated adenomatous carcinomas

G 2. *differentiated adenomatous carcinomas* with partly solid areas,

G 3 predominantly solid or entirely undifferentiated carcinomas.

Stage II The carcinoma has involved the corpus and the cervix.

Stage III The carcinoma has extended outside the uterus but not outside the true pelvis.

Stage IV The carcinoma has extended outside the true pelvis or has obviously involved the mucosa of the bladder or rectum. A bullous oedema as such does not permit allotment of a case to Stage IV

Carcinoma of the Vagina

Cases should be classified as carcinoma of the vagina when the primary site of the growth is in the vagina. Tumours presenting in the vagina as secondary growths from either genital or extragenital sites should be excluded from registration.

A growth that has extended to the portio and reached the area of the external os should always be allotted to carcinoma of the cervix

It has been proposed that cases which according to the above classification are tumours of low potential malignancy i.e. Ib, IIb, IIIb, and IVb, should be called borderline cases. The Cancer Committee of the Federation cannot accept this term, especially as histologically unquestionably benign tumours of papillary nature may give rise to implantation of metastases which spontaneously disappear after removal of the primary growth. Such tumours should not be included among cases of Ib IIb, IIIb or IVb The Cancer Committee is aware of the fact that ovarian neoplasms allotted to the group low potential malignancy" may be of different biological behaviour At present there is, however no method to sub-divide these cases.

In ovarian tumours it is desirable to have a clinical stage-grouping like those in the other malignant tumours in the female pelvis. Sometimes it is impossible to come to final diagnosis by inspection or palpation or by any of the other methods recommended for clinical staging of carcinoma of the uterus and vagina. Therefore, the Cancer Committee of the Federation has recommended that the clinical staging of ovarian cancer should be based on clinical examination as well as on findings at laparotomy In some cases of malignant tumour in the pelvis or abdomen the condition of the patient does not permit explorative laparotomy and they can thus not be clinically staged in detail. For the presentation of therapeutic results it is necessary however to receive information concerning the number of such patients who are thought to have an ovarian malignant tumour They should be reported in the group "Special category In 1964 the General Assembly of FIGO approved on stage-grouping of ovarian carcinoma based on findings at clinical examination and laparotomy In 1966 the TNM Committee of the UICC proposed a TNM classification of ovarian cancer This classification is, however not based on studies of large consecutive series of cases. It is under trial in the years 1967 1971

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Stage-grouping for primary ...
(To be used from January ...

Stage I Growth limited to the ovaries.

Ia. Growth limited to one ovary: no ascites (i) capsule ruptured, (ii) capsule not ruptured.

Ib· Growth limited to both ovaries no ascites (i) capsule ruptured, (ii) capsule not ruptured.

Ic: Growth limited to one or both ovaries ascites present with malignant cells in the fluid (i) capsule ruptured, (ii) capsule not ruptured.

Stage II Growth involving one or both ovaries with pelvic extension.

IIa. Extension and/or metastases to the uterus and/or tubes and/or other ovary

IIb· Extension to other pelvic tissues.

Stage III Growth involving one or both ovaries with wide-spread intraperitoneal metastases.

Stage IV Growth involving one or both ovaries with distant metastases.

Special category Unexplored cases which are thought to be ovarian carcinoma.

Stage III	*Stage IV*
T3 N0 M0	T1 N3 M0
T3 N1 M0	T2 N3 M0
T3 N1 M0	T3 N3 M0
T3 N2 M0	T4 N3 M0
T1 N2 M0	T4 N0 M0
T2 N2 M0	T4 N1 M0
	T4 N2 M0
	All other conditions containing M 1a or M 1b

If cytology or histology of lymph nodes reveal malignant cells the symbol + (plus) should be added to N if such examinations do not reveal malignant cells the symbol − (minus) should be added to N

Carcinoma of the Urethra

Cases should be classified as carcinoma of the urethra when it is evident that the primary site of the growth is in the urethra.

It is important to separate cases of carcinoma at the external urethral os from cases originating deeper in the urethra. In 1968 the TNM Committee of the UICC proposed a TNM classification of carcinoma of the urethra in women. For the present the Cancer Committee of the Federation has not proposed a stage-grouping for carcinoma of the urethra.

Carcinoma of the Ovary

Ovarian carcinoma is a common malignant tumour It can not be regarded as an entity Therapeutic statistics on ovarian cancer are of limited value if attention is not paid to the histological type of the growth. Experience has shown that there is no clear correlation between clinical and histological malignancy in ovarian tumours. This holds valid for various types of neoplasms but especially for epithelial tumours, granulosa cell tumours, and virilizing tumours.

The Cancer Unit of the WHO has set up a special Reference Centre for histo-pathological classification of various ovarian tumours. The Cancer Committee of FIGO is pleased to have a close collaboration with this Reference Centre.

In 1961 the Cancer Committee of FIGO proposed a histo-pathological classification of the common primary epithelial ovarian tumours. This proposal has been slightly modified. The Reference Centre of the WHO has not yet presented their final recommendation but it is important to know that, in principle, the Reference Centre and the FIGO are working along the same lines.

Histo-pathological classification of ovarian tumours

Cases of germ cell tumours, hormonal producing neoplasms, and metastatic carcinomas should be excluded from therapeutic statistics on ovarian epithelial tumours.

Histological classification of the common primary epithelial tumours of the ovary (To be used from January 1 1971)

I. *Serous cystomas*

(*a*) Serous benign cystadenomas.

(*b*) Serous cystadenomas with proliferating activity of the epithelial cells and nuclear abnormalities but with no infiltrative destructive growth (low potential malignancy)

(*c*) Serous cystadenocarcinomas.

II *Mucinous cystomas*

(a) Mucinous benign cystadenomas.

(*b*) Mucinous cystadenomas with proliferating activity of the epithelial cells and nuclear abnormalities but with no infiltrative destructive growth (low potential malignancy)

(*c*) Mucinous cystadenocarcinomas.

III. *Endometrioid tumours* (similar to adenocarcinomas in the endometrium)

(*a*) Endometrioid benign cysts.

(*b*) Endometrioid tumours with proliferating activity of the epithelial cells and nuclear abnormalities but with no infiltrative destructive growth (low potential malignancy)

(*c*) Endometrioid adenocarcinomas.

IV *Mesonephric tumours*

(*a*) Benign mesonephric tumours.

(*b*) Mesonephric tumours with proliferating activity of the epithelial cells and nuclear abnormalities but with no infiltrative destructive growth (low potential malignancy)

(*c*) Mesonephric cystadenocarcinomas.

V *Concomitant carcinoma unclassified carcinoma* (tumours which cannot be allotted to one of the groups I II III, or IV)

It has been proposed that cases which according to the above classification are tumours of low potential malignancy i.e. Ib IIb, IIIb, and IVb, should be called borderline cases. The Cancer Committee of the Federation cannot accept this term, especially as histologically unquestionably benign tumours of papillary nature may give rise to implantation of metastases which spontaneously disappear after removal of the primary growth. Such tumours should not be included among cases of Ib, IIb IIIb, or IVb The Cancer Committee is aware of the fact that ovarian neoplasms allotted to the group "low potential malignancy" may be of different biological behaviour At present there is, however no method to sub-divide these cases.

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Stage-grouping for primary [illegible]
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Ib Growth limited to both ovaries, no ascites (i) capsule ruptured, (ii) capsule not ruptured.

Ic Growth limited to one or both ovaries; ascites present with malignant cells in the fluid (i) capsule ruptured, (ii) capsule not ruptured

Stage II Growth involving one or both ovaries with pelvic extension.

IIa. Extension and/or metastases to the uterus and/or tubes and/or other ovary

IIb Extension to other pelvic tissues.

Stage III Growth involving one or both ovaries with wide-spread intraperitoneal metastases.

Stage IV Growth involving one or both ovaries with distant metastases.

Special category Unexplored cases which are thought to be ovarian carcinoma.

Stage III nent
T 3 N 0
T 3 zechoslovak Society for Endocrinology and T 'Society of Gynaecologists of the Czechoslovak Medical Society J E Purkyně are organizing a *Symposium on Gonadotropic Hormones in Endocrine Disorders of Human Reproduction* with international participation to be held in Smokovec, High Tatras, Czechoslovakia from September 15 to 17 1971

Papers can be read in English and German. Participants are requested to send in preliminary registration by December 31 1970. Summaries of papers are to be submitted by April 30 1971

For information and registration please write to the Secretary General

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Acta Obstet Gynec Scand 50: 9–16, 1971

MENSTRUAL BLEEDING WITH INTRAUTERINE CONTRACEPTIVE DEVICES

Elgil Guttorm

From Obstetrical Department A and Gynaecological Department I (Heads: Dyre Trolle and Børge Sørensen), Rigshospitalet University of Copenhagen, Denmark

Abstract. Ninety women with an intrauterine device (IUD) in place measured their menstrual flow maximum of 16 months after the insertion.

The average loss was 88.0 ml. Those who had subjective menorrhagia after the insertion (group II) had heavier bleeding than those without (group I), 116.7 ml as compared with 78.7 ml.

Menorrhagia (blood loss exceeding 80 ml) occurred in 42.6 of group I and in 63.6% of group II. The rate for the entire series was 47.7 (Table III).

16% had subjective menorrhagia although the blood loss was less than 80 ml, the upper limit of normal bleeding. On the average, these patients lost 53.1 ml (Table III).

The Hb level fell after the insertion of the IUD from 13.5 g/100 ml to 12.8 g/100 ml. The decrease was more marked in group II, and in 11% the level dropped by more than 1.5 g/100 ml.

The incidence of anaemia (Hb below 12.0 g/100 ml) increased from 3% to 16% after the insertion of the device. *For groups I and II the increase in the rate was* from 2% to 10% and from 9 to 32% respectively. All considered, 4–5-fold increase occurred (Table V).

The Hb level as well as the menstrual blood loss were the same whether they were measured months or 2–16 months after the insertion.

The distribution of the bleeding on the individual days of the period changed. 20 women measured the loss without and 90 with the IUD.

In the group without IUD 73% of the total loss occurred during the first 2 days of the period and 90% during the first 3 days. In the women with IUD the distribution was 44% and 74%, the 90 not being reached until after the 4th day (Tables VI and VII).

Twenty women measured the menstrual loss both before and after the insertion of the device. In all the menstrual flow became heavier increasing by an average of from 35.5 to 73.1 ml (106%). The increase was most marked in women whose menstrual flow had been scanty before the insertion of the device (Table VIII). The mean values of the menstrual bleeding measured during the 1st, 2nd, 3rd, and 4th period after the insertion were practically the same, from 65.8 to 82.. ml, but always significantly greater than prior to the insertion. There was no tendency for the menstruation to return to normal level (Table IX).

The increase in the use of absorptive material during the menstrual periods was not in step with the bleeding, being only 28%, despite an increase of 106% in the blood loss, and 25% of the women had an unchanged or even decreased consumption of absorptive material after the insertion of the IUD.

In intrauterine contraception bleeding disturbances are common, and 5–10% of the devices have to be removed for this reason, irrespective of the type used.

The first menstrual periods after the insertion are reported to be heavier and longer-lasting than usual. The reported incidence of this increase has differed within wide limits, from 31% (Ohlsrup & Guttorm, 1967) to 64% (Guttorm, 1969). Fulton et al. (1967) found a decrease of more than 10% in the Hb level in 23% after insertion of Gynekoll.

The subjective description of the menstrual flow *as well as its influence upon the haemoglobin* level indicate an increase of the menstrual blood loss. To demonstrate the change in the bleeding pattern the menstrual flow was measured in women who had been wearing IUD for varying period of time. In a small number of cases the *flow was also measured before* the insertion of the IUD.

METHODS

A number of different methods have been advocated for measuring the menstrual blood loss. For large-scale clinical use the one described by Hallberg & Nilsson (1964

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T 3 zechoslovak Society for Endocrinology and T Society of Gynaecologists of the Czechoslovak Medical Society J E. Purkyně are organizing a *Symposium on Gonadotropic Hormones in Endocrine Disorders of Human Reproduction* with international participation to be held in Smokovec, High Tatras, Czechoslovakia from September 15 to 17 1971

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MENSTRUAL BLEEDING WITH INTRAUTERINE CONTRACEPTIVE DEVICES

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Abstract. Ninety women with an intrauterine device (IUD) in place measured their menstrual flow maximum of 16 months after the insertion.

The average loss was 89.0 ml. Those who had subjective menorrhagia after the insertion (group II) had heavier bleeding than those without (group I), 116.7 ml as compared with 78.7 ml.

Menorrhagia (blood loss exceeding 80 ml) occurred in 42.6% of group I and in 63.6% of group II. The rate for the entire series was 47.7% (Table III).

36% had subjective menorrhagia although the blood loss was less than 80 ml, the upper limit of normal bleeding. On the average, these patients lost 53.1 ml (Table III).

The Hb level fell after the insertion of the IUD from 13.5 g/100 ml to 12.8 g/100 ml. The decrease was more marked in group II, and in 11% the level dropped by more than 1.5 g/100 ml.

The incidence of anaemia (Hb below 12.0 g/100 ml) increased from 3% to 16% after the insertion of the device. For groups I and II the increase in the rate was from 2% to 10% and from 9% to 32% respectively. All considered, a 4–5-fold increase occurred (Table V).

The Hb level as well as the menstrual blood loss were the same whether they were measured within months or 2–16 months after the insertion.

The distribution of the bleeding on the individual days of the period changed. 20 women measured the loss without and 90 with the IUD.

In the group without IUD 73% of the total loss occurred during the first 2 days of the period and 90% during the first 3 days. In the women with IUD the distribution was 44% and 74%, the 90 not being reached until after the 4th day (Tables VI and VII).

Twenty women measured the menstrual loss both before and after the insertion of the device. In all the menstrual flow became heavier, increasing by an average of from 35.5 to 73.1 ml (106%). The increase was most marked in women whose menstrual flow had been scanty before the insertion of the device (Table VIII). The mean values of the menstrual bleeding measured during the 1st, 2nd, 3rd, and 4th period after the insertion are practically the same, from 65.8 to 82.2 ml, but always significantly greater than prior to the insertion. There was no tendency for the menstruation to return to normal level (Table IX).

The increase in the use of absorptive material during the menstrual periods was not in step with the bleeding, being only 28% despite an increase of 106% in the blood loss, and 25% of the women had an unchanged or even decreased consumption of absorptive material after the insertion of the IUD.

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The subjective description of the menstrual flow as well as its influence upon the haemoglobin level indicate an increase of the menstrual blood loss. To demonstrate the change in the bleeding pattern the menstrual flow was measured in women who had been wearing IUD for varying period of time. In a small number of cases the flow was also measured before the insertion of the IUD.

METHODS

A number of different methods have been advocated for measuring the menstrual blood loss. For large-scale clinical use the one described by Hallberg & Nilsson (1964)

Table I *Distribution of series (no of measured menstrual periods)*

Group I Women without subjective menorrhagia
Group II Women with subjective menorrhagia

Group	Interval after insertion			No of women
	< 6 months	> 6 months	Total	
I	114	24	138	68
II	20	25	45	22
Total	134	49	183	90

was found to be best suited and was selected for the present study Its principle will be briefly described: To collect menstrual blood sanitary tampons and towels were used and subjected to extraction with 5% sodium hydroxide for 20 hours The haemoglobin will be converted into alkaline haematin. The concentration is determined spectrophotometrically and the total quantity calculated. If the Hb level is known, it is easy to calculate the blood volume represented by the haematin.

The values must be considered minimum values, as some waste is bound to occur on changing, especially in cases with heavy bleeding.

The menstrual blood was collected in Mimosept towels and OB tampons kindly supplied by AB Mölnlycke All types of absorptive material are not suitable as some include dyes which affect the measurement.

The women were provided with towels or tampons and the necessary equipment for storing the used absorptive material. The material was collected every day so that information was obtained about the total blood loss as well as its distribution on the individual days of the period.

The total menstrual loss in each woman, when several periods had occurred, will be given as the mean value of these periods. All the data were collected for computer analysis, and all calculations were performed by NEUCC with the assistance of Chr Rovsing Ltd., computer adviser to Rigshospitalet.

MATERIAL

The series comprises women who were fitted with IUD in the Department and who were later seen for follow up, with or without complaints on account of the IUD

The total series of 90 women was divided into two groups:

Group I. Women without subjective menorrhagia.
Group II Women with subjective menorrhagia.

These women measured a total of 183 menstruations, from 1 to 4 each. The longest interval after the insertion was 16 months.

The distribution of the two groups is shown in Table I.

In 20 women the menstrual flow was measured before as well as after the insertion of the IUD All reported that the menstruation had been normal prior to the insertion. None developed subjective menorrhagia after the insertion of the IUD and thus all accidentally fell into group I.

Devices of the following types were used:

Birnberg bow No. 5 78
Birnberg bow No. 6. 4
Antigon: 8

The majority of the women were about 30 years of age The youngest was 18 and the oldest 47 years. Most of them had a history of 2 or more deliveries, including 10 by Caesarean section (cf. Table II).

RESULTS

The menstrual flow varied widely from individual to individual but was fairly constant in each patient. An impression of the individual variations is given in Fig 1 which shows the distribution of the women in relation to the menstrual flow for the entire series as well as for each of the two groups. The minimum value is 21.8 ml and the maximum 249 7 ml

The mean value with the standard error of mean is, for the entire series (90 women) 88.0 ml $\pm$ 4 8 range as just stated. For group I (68 women) it was 78 7 ml $\pm$ 4 1 and for group II (22 women) 116 7 ml $\pm$ 13 3

As might be expected, women with subjective menorrhagia had, on the average a heavier flow The difference is significant ($t = 2.74$ $P < 0.02$)

The reported upper limit of normal for the quantity of the menstrual loss has differed widely On the basis of a comprehensive population study in Gothenburg, Sweden, Hallberg et al. (1966) found it to be 80 ml using the same measuring technique. This limit was selected also for the present study as the conditions of living and environment must be presumed to be largely the same in Copenhagen and in Gothenburg

If menorrhagia is defined as menstrual bleeding in excess of 80 ml 4_6% of the women in

Table II *Distribution by age and parity*

Age	20 y	1 30 y	31–40 y	41 y	Total
> Gra I					
Para 0	0	1	0	0	1
Para I	2	9	1	0	12
Para II	1	29	11	1	4
> Para III	1	70	13	1	35
Total	4	59	25		90

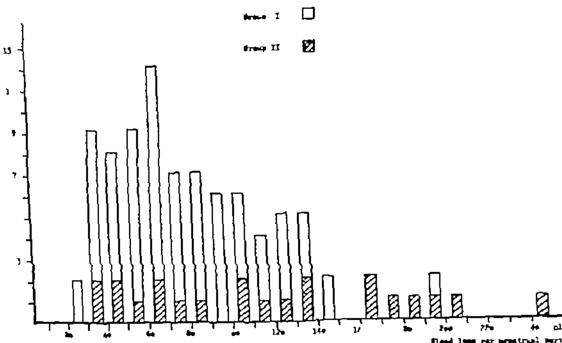


Fig. 1. Quantity of menstrual blood loss distributed in the tw groups. *N* = number of women—total 90.

group I and 63.6% of those in group II had menorrhagia. The rate f r the entire series is 47 7% (cf Table III).

Calculation of the mean value for those women who did not have menorrhagia gave the same result whether or not they reported subjectively increased menstrual flow viz. 54 7 ml ± .4 and 53 1 ml ± 5 4 respectively

Among women who did have menorrhagia, on the other hand, the mean value was higher f r those who reported a subjectively increased menstrual flow than for those who did not, viz. 153.1 ml ± 12.5 and 111.0 ml ± 4 6 respectively

It is striking that in 36% (8/22) of the women who reported a subjectively increased menstrual flow the loss was 80 ml or less. The explanation is no doubt that the flow has increased after the insertion of the IUD but without exceeding the upper limit of normal

It is well-known that menorrhagia increases the risk of anaemia, and indeed anaemia was more commonly found in group II than in group I. If the criterion of anaemia is fixed at Hb level below 12 g/100 ml, the incidence was 10% in group I and 32% in group II (cf. T ble IV).

That the menstrual bleeding bears the sole responsibility for the anaemia is indicated by th finding that the menstrual bleeding was significant higher in women with than without anaemia. Th applied to group I as well as group II (cf. Tab IV). The mean values for women with anaem were 117 1 ml ± 18.9 and 163 7 ml ± .2.3 respe tively and for women without anaemia 74 1 r ± 3.8 and 94 8 ml ± 11 4 respectively

In 89 of the women the haemoglobin level ha also been determined less than 1 month befo the IUD was inserted. After the insertion the H

Table III. *Cases of menorrhagia (> 80 ml) in grou I and II*

Group I Women without subjective menorrhagia
Group II Women with subjective menorrhagia

	Group I		Group II		
Menstrual loss (ml)	No.	Aver blood loss ± S E M. per menstrual period	No	Aver blood loss ± S.E.M. per menstrual period	To
< 80 ml	39	54 7 ml ± 2.4	8	53 1 ml ± 5 4	47
80 ml	29	111.0 ml ± 4 6	14	153 1 ml ± 12.5	43
Total	68	78.7 ml ± 4 1	22	116 7 ml ± 13.3	90

Table IV *Incidence of anaemia and average blood loss per menstrual period*

Group I Women without subjective menorrhagia
Group II Women with subjective menorrhagia

	No of women	%	Blood loss (ml) Per menstrual period ± S.E.M
Group I			
Hb % < 12.0 g	7	10 4	117 1 ml ± 18.9
Hb % ≥ 12.0 g %	60	89 6	74 1 ml ± 3.8
	67	100 0	
Group II			
Hb % < 12.0 g %	7	31 8	163 7 ml ± 27.3
Hb % ≥ 12.0 g %	15	68.2	94 8 ml ± 11 4
	22	100 0	

level was determined at the time of calculating the menstrual blood loss, a maximum of 16 months after the insertion Comparison of the mean values ± standard error of mean before and after the insertion of the IUD showed a decrease of from 13.5 g/100 ml ± 0 1 to 12.8 g/100 ml ± 0 1 The difference is significant ($t = 5\ 6$ $P < 0\ 001$) For groups I and II the decrease was from 13.5 g/100 ml ± 0 1 to 12.9 g/100 ml ± 0 1 and from 13.3 g/100 ml ± 0.2 to 12.3 g/100 ml ± 0.2 respectively

The maximum drop was 3 2 g/100 ml and in 11% of the cases (10/89) it was ≥ 1 5 g/100 ml which exceeds the maximum error of measurements.

If anaemia is defined as a Hb level below 12 g/100 ml the incidence in the entire series prior to the insertion was 3% and after the insertion 16% (cf Table V) For group I the incidence was

Table V *Cases of anaemia (< 12.0 g/100 ml) before and after insertion of IUD*

Group I Women without subjective menorrhagia
Group II Women with subjective menorrhagia
Difference significant ($\chi^2 = 10\ 7$ $P < 0\ 01$)

	Anaemia before insertion		Anaemia after insertion		Total women
	No		N		
Group I	1	2	7	10	67
Group II	2	9	7	32	22
Total	3	3	14	16	89

Table VI *Percentage distribution of total blood loss per day of the menstrual period for women with and without IUD*

Day of menstrual period	Women with IUD (total 90) Entire series	Blood loss > 80 ml	Blood loss < 80 ml	Women without IUD (total 70) All bleeding < 80 ml
1	12.2	9 8	14.3	28 7
2	31.9	31 3	32.6	44.6
3	29 7	32.2	27 4	16.4
4	16.2	17 7	14 7	6.8
5	6.3	6.1	6.5	2.4
6	2.0	1 7	2.3	0 8
7	1 7	1.2	2.1	0.4

2% and 10% respectively and for group II 9% and 32%

In other words, the number of women with anaemia was 4–5 times larger after the insertion of the IUD irrespective of whether the women reported subjective menorrhagia.

It has often been claimed that only the first menstrual period after the insertion is increased. If this is correct, the mean Hb level measured less than 2 months after the insertion would be expected to be lower than that measured later

To elucidate this aspect, the mean values were calculated for the Hb levels determined within 2 months and from 2–16 months after the insertion of the device.

For the entire series the results were 12.8 g/100 ml and 12.7 g/100 ml respectively and for group I 12.9 g/100 ml and 12.8 g 100 ml

Calculation in the same way of the mean values for the menstrual flow gives for group I 77.5 ml and 73 5 ml respectively The mean values for Hb level and blood loss in group II will not be included, as the number of menstrual flows measured less than 2 months after the insertion is too small.

According to the present findings the Hb level as well as the menstrual flow appear to remain unchanged, not showing the expected increase in Hb or decrease in menstrual flow

The distribution of the menstrual flow on the individual days of the period was also investigated. Table VI presents the percentage distribution of the total loss on the individual days of the period It is apparent that in women wearing a device the flow is heaviest on the 2nd, 3rd and 4th day

Table VII. *Average blood loss per day of menstrual period in ml with and without IUD*

Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	≥ Day 7	Total
Women with IUD							
9 5	27.8	27 1	15.1	5 4	1.6	1.2	87 7
Women without IUD							
10 2	15.9	5 9	2.2	0.9	0.3	0.1	35.5

This applies both when the bleeding is above and below 80 ml. However the bleeding was heavier on the 3rd and 4th days when the menstrual loss exceeded 80 ml, but the difference is not significant.

In 20 women the menstrual flow was measured before the IUD was inserted. It was found to be heaviest on the 1st and 2nd days (cf. Tables VI and VII).

Comparison of the mean values for each day of the period in women with and without IUD showed that this difference is significant ($\chi^2 = 499$, $P < 0.0005$, cf. Table VII).

Thus, in women without IUD 73.3% of the total loss occurs on the first 2 days of the menstrual period and 89 7% in the course of the first 3 days. After the insertion of the IUD the distribution was 44 1% and 73.8% and 90% was not reached until the 4th day.

The women often notice this altered bleeding pattern, stating that the bleeding cannot get going, the menstruation does not properly break through and "there is slight bleeding before and after each period".

Apart from the distribution of the flow on the individual days of the menstrual period, the *quantity of the bleeding* in these 20 women was compared before and after insertion of the IUD. The women measured 1 or 2 menstrual losses both before the insertion and a maximum of 5 months after. None of them reported subjective menorrhagia *after the insertion of the IUD* and thus accidentally fell in to group I.

The result is listed in Table VIII which shows that the average blood loss had more than doubled after the insertion, increasing from 35.5 ml ± 6.5 to 73 1 ml ± 8 3. This *difference is significant* ($t = 3.56$, $P < 0.001$). It should be mentioned that in all 20 women each individual menstrual flow after the insertion of the IUD was heavier than before.

Table VIII. *Average blood loss per menstrual period before and after insertion of IUD and relation between the change and the quantity of the menstrual loss before the insertion*

No. of cases	Average blood loss per menstrual period ± S.E.M.		Average change	
	Before insertion	After insertion	(ml)	(%)
14	0 to 40 ml	62.5 ± 9 5 ml	41.9 ± 9 2	202.6
4	41 to 80 ml	89 1 ± 11.5 ml	30 6 ± 11 7	52.3
1	> 80 ml	140.3	13 2	10.4
20	35.5 ± 6 5	73 1 ± 8.3	37 6 ± 7 1	106.1

If the menstrual losses prior to the insertion are grouped by quantity (cf. Table VIII), we find that women who had the scantiest flow prior to the insertion *exhibit the greatest percentage as* well as absolute increase. There is significant difference in the loss before and after the insertions in the groups, from 0 to 40 ml ($t = 4.22$, $P < 0.001$) and 41 to 80 ml ($t = 2.51$, $P < 0.05$).

All 20 women reported that their menstruation was normal prior to the insertion and that the first menstruation after the insertion was somewhat increased but later returned to normal.

To ascertain whether this subjective statement was correct, the mean value was calculated *for* menstrual periods measured as the first, second, etc. after the insertion. The results are presented in Table IX.

The variation of the mean values was slight *within the first* 6 months after the insertion, from 65.8 ml to 82.2 ml. The second menstruation was somewhat heavier than the others, but the difference is not significant. On the other hand, all the values are significantly higher than prior to

Table IX. *Menstrual loss before insertion of IUD compared with that immediately after*

Menses, in relation to insertion of IUD	No. of women	Aver. blood loss per menstrual period ± S.E.M.
Before insertion	20	35 5 ± 6 5
After insertion		
1st period	11	66 6 ± 9 8
2nd period	10	82.2 ± 17.6
3rd period	5	68 6 ± 22 5
≥ 4th period	6	65.8 ± 7 4

the insertion. This indicates that the change in the menstrual pattern is fairly permanent, and that it does not return to the normal level within the first 6 months.

The number of absorptive towels or tampons used had been on average 14 6 before and 18.5 *after the insertion of the IUD* an increase of 28 % Out of the 20 women 15 had an increased use one unchanged, while 4 used less than prior to the insertion.

DISCUSSION

Hallberg & Nilsson (1964) have demonstrated that the quantity of menstrual blood lost is so constant in each individual woman that a measurement of one period affords a satisfactory impression of this particular woman's bleeding level. The same applies to women wearing an IUD in whom two or more menstrual periods have been measured Therefore, we have also included women in whom only a single measurement had been obtained.

The average menstrual loss in the entire series was 88 ml, or more than twice that found by Hallberg et al. (1964) in a population study in Gothenburg, viz. 43 4 ml. No person in their series was wearing an intrauterine contraceptive device.

In women without anaemia or subjective menorrhagia and who were on the whole in good health, we found—like Hallberg et al (1964)—a lower average flow but still about twice that reported by Hallberg et al viz. 74 1 ml as compared with 38.5 ml (Table IV)

Among the 20 women of group I in whom the menstrual loss was measured both before and after the insertion of the IUD we also found a more than two-fold increase in the blood loss. The mean loss (35.5 ml) before the insertion was of the same magnitude (38.5 ml) as found by Hallberg et al. (1964) in healthy women with normal menstruation.

Accordingly it is beyond doubt that there is a real increase in the menstrual loss of 100 due exclusively to the IUD

It is generally believed that only the first menstrual period after the insertion is increased. *If so the mean value of the Hb readings* within the first 2 months after the insertion would be expected to be decreased and later Hb readings higher in the same range as prior to the insertion. Moreover the menstrual loss measured less than 2 months after the insertion would be expected to be heavier than the losses measured later

However this is not so A significant fall in the Hb level was found after insertion of the *IUD* but the mean value of the *Hb* level was the same within the first 2 months after the insertion as 2–16 months after Similarly the average menstrual loss remained unchanged after the insertion.

In the 20 women whose menstruation was measured both before and a maximum of 5 months after the insertion of the IUD the flow also remained increased, and all values were significantly higher than before the insertion.

These findings indicate that the menstrual loss remains unchanged at a higher level at least during the first 6 months.

The explanation of the discrepancy between the subjective and objective assessment of menstruation is that factors other than the bleeding influence the use of absorptive material. There may have been a considerable increase in the menstrual flow although the woman has not noticed it, as is apparent from the fact that 25 % used neither more nor less and that on the whole the increase in the use of absorptive material was only 28 % although the bleeding had, on average, doubled.

Hallberg et al. (1966), Rybo (1966) and Jacobs & Butler (1965) have reported that the upper limit of normal menstrual flow is 80 ml and that above this limit there is an increased risk of anaemia due to the loss of iron through the menstrual blood.

If this limit is accepted, 48 of women wearing an IUD have menorrhagia. The rate in group I is 4_6 % and in group II 63 _ In the Gothenburg study Rybo (1966) reported 12.? % for women without IUD

In other words, menorrhagia is 4–5 times as common *in women with an IUD as in those of* Rybo's (1966) study This is in keeping with the fact that in the present study the incidence of anaemia increased 4–5-fold after the insertion of the IUD

The reports on the incidence of anaemia have differed a great deal. Rybo (1966) found 6 in female industrial workers. Hallberg et al (1964) reported that with a limit at 11.5 g 100 ml it was 15–_5 varying with age highest during

the fertile age and at a maximum around the age of 30.

The lower incidence (3% Table V) found in the present series prior to the insertion of the IUD is no doubt due to a different age, social, and environmental distribution. Although the incidence of anaemia among women wearing IUD is not definitely higher than that found by others in women without IUD (Hallberg et al., 1964), the author feels justified in maintaining that it increased 4–5-fold, as the Hb level was measured by the same technique in the same woman both before and after the insertion of the IUD

This assertion is also supported by the finding that the number of cases of menorrhagia was about four times larger than in the Gothenburg series and that the increased bleeding, caused by IUD is responsible for the increased incidence of anaemia.

Rybo (1966) investigated the influence of age, parity and interval after parturition upon the menstrual flow He found 15-year-old girls to have scantier and women over 45 to have heavier menstrual flow but from the age of 18 until 45 there was no difference. Young nulligravidae had somewhat scantier flow but otherwise parity had no influence upon the amount of the flow The flow was independent of the interval after parturition.

All the women in the present series were between 18 and 45 years of age and none was a nulligravida. Thus, differences in age parity and interval after parturition cannot have caused the heavier average menstrual flow

The menstrual loss is not evenly distributed on the individual days of the period. Baldwin et al. (1961) found that 76% of the total loss occurred on the first days, Callard et al. (1966) 70% and Rybo (1966) 78%

Rybo (1966) showed, moreover slight difference in the distribution of losses of more and of less than 80 ml, but this difference was not significant. Thus, among women having losses of less than 80 ml, he found 79% of the total loss to occur in the course of the first 2 days and 90% in the course of the first 3 days. In women losing more than 80 ml the corresponding values were 69% and 86%

Those women in the present series in whom the menstrual flow was measured prior to the insertion of the IUD showed the same distribution as found by Rybo (1966), viz. 73% and 90% respectively In women wearing an IUD, on the other hand, 44% of the total loss occurred in the course of the first 2 days, 74% in the course of the first 3 days, and 90% had not been lost until the 4th day This difference in the distribution is significant.

In the case of menstrual bleeding of less than 80 ml women wearing an IUD lose 47% of the total flow on the first 2 days of the menstrual period and 74% in the course of the first 3 days. If the loss exceeds 80 ml these values are 41% and 74% respectively

Comparison of a menstrual loss above and below 80 ml showed in women wearing an IUD tendency to a later maximum flow when the total loss exceeded 80 ml, but the difference is not significant (cf. Table VI and p. 12). This is the same as found by Rybo (1966) in women without an IUD

Thus, the increased menstrual flow in women wearing an IUD cannot explain the significant difference in the distribution of the flow on the individual days of the menstrual period after insertion of an IUD.

Therefore, the change in the distribution of the flow on the individual days of the menstrual period can hardly be due to anything but the inserted IUD

CONCLUSIONS

The present study has shown that in women wearing intrauterine contraceptive devices (IUD) the menstrual flow will be doubled, increasing most in those who have had a normal or scanty loss prior to the insertion of the IUD

The increased menstrual flow appears to persist through the first 6 months after the insertion, without any tendency to return to normal.

Menorrhagia (a loss exceeding 80 ml) and anaemia (Hb level below 12.0 g/100 ml) are 4–5 times more common after the insertion of an IUD

The amount of blood loss on the individual days of the period changes, so that it is heaviest on the 2nd and 3rd day as compared with the 1st and 2nd day of the period in women without an IUD.

It must be recommended that a frequent check be kept on the haemoglobin level and that iron supplements be given if necessary to women with

IUD because of the increased menstrual bleeding. This also applies to women who state that the menstrual flow is normal as the woman does not always notice that it has increased.

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CANCER OF THE UTERINE CERVIX IN A KOREAN BIOPSY SERIES

With a Study of the Age Distribution in 1 889 Cases of Chronic Cervicitis, Dysplasia, Intra-epithelial and Invasive Epidermoid Carcinoma

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Abstract. Invasive cervical carcinoma is the most commonly observed primary form of cancer in Korean females. This tumour constituted 37.6% of 1 820 malignant tumours of known primary site encountered in a Korean surgical series at the Scandinavian-sponsored National Medical Center in Seoul from December 1958 to August 1967. The age at diagnosis of invasive cervical cancer averaged 43.6 years, and the estimated population peak incidence was also within the fifth decade. Ninety-five per cent of the cases were of the epidermoid type, the range of age among patients with adenocarcinoma was significantly greater than in those with epidermoid cancer. The age distribution of 1 889 cases of simple chronic cervicitis, squamous-cell dysplasia, intra-epithelial neoplasia and invasive epidermoid carcinoma is presented and discussed. The observations in 1 033 cases of progressive epithelial abnormalities are consistent with a linear regression of age at diagnosis on histological grade of epithelial alterations ranging from slight dysplasia to late invasive epidermoid cancer. Such an interrelation constitutes one among several circumstantial pieces of evidence that the morphological evolution of invasive epidermoid carcinoma passes through a spectrum of epithelial abnormalities. The present results support the concept of a step-wise progression in epidermoid cervical carcinogenesis.

Cervical carcinoma is one of the commonest forms of malignant disease in most Asiatic countries (29, 39), and it is the most frequently encountered primary form of cancer in Korean females (20, 21, 38, 41).

The relationship between intra-epithelial and invasive cervical cancer is generally accepted, but a number of recent clinical, cytological and histological studies have been concerned with whether chronic irritative and inflammatory cervical lesions showing basal-cell hyperplasia and advancing grades of squamous-cell dysplasia could be premalignant. Such a spectrum of epithelial abnormalities may represent a chronological progression to carcinoma (2, 4, 11, 12, 23, 27, 28, 30, 40).

On comparing the age incidence in advancing grades of cervical dysplasia, intra-epithelial and invasive carcinoma, Mali et al. (23) found a gradual and definite increase in the mean age in a large Indian series. The present communication deals in particular with the age distribution of cases of histologically graded epithelial abnormalities of the uterine cervix, diagnosed in 1 889 South Korean women who were examined at the National Medical Center (NMC) in Seoul.

MATERIAL AND METHODS

The NMC in Seoul is a Korean post-graduate training hospital, sponsored since 1958 by the three Scandinavian countries Denmark, Norway and Sweden. The Center has a special pathology laboratory. Scandinavian pathologists have been in charge of this department from the opening of the hospital in 1958 until the end of 1967.

A total of 10 598 cases of histologically demonstrable disease in Korean women was encountered among 23 056 surgical specimens examined from December 1958 to August 1967. Cancer of the female sex organs (including breast) made up 1 044 cases, or no less than 55.4% of a total of 1 885 malignant tumours in women.

Uterine cancers comprised 815 (43.2%) of these neoplasms. Invasive carcinoma of the uterine cervix was by far the most commonly encountered primary form, totalling 685 cases (65.6%) of all primary genital cancer in females. This form of cancer alone made up 36.3% of all malignant diseases in women, and 37.6% of the malignant tumours of known primary site in this sex (1 820 cases).

The distribution of the total female population by age was available for the 1957–65 period, and was cal-

IUD because of the increased menstrual bleeding. This also applies to women who state that the menstrual flow is normal as the woman does not always notice that it has increased.

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An overall analysis failed to disclose any significant differences between the three group means. The last column in Table II gives the overall age distribution of the non-malignant cervical conditions (38.6 ± 8.5 years).

Epidermoid cancer series (Groups 3 4 and 5 713 cases)

Table III shows the age distribution in each of the three sub-series of epidermoid cervical carcinoma. An overall highly significant heterogeneity was present among the three age means. The intra-epithelial average proved to be definitely below that of the pooled series of invasive carcinoma. The difference between early and late invasive cancer was, however short of significance at the 5 % level, although being a borderline case (0.05 < P < 0 10)

The 95 % confidence limits for the true difference in mean age between intra-epithelial and invasive epidermoid carcinoma as whole ranged from 2.8 to 5.0 years.

Non-malignant conditions versus pidermoid cancer series

The pooled mean of the non-malignant lesions was not significantly different from that of intra-epithelial carcinoma. An additional test of all comparisons between the six group means revealed that the only significant step-specific difference was that between intra-epithelial and early invasive carcinoma (2.6 years, Table III). The 95 % confidence limits for this difference were estimated at 1.2 and 4.0 years.

However gradual increase in mean age is

Table II. *Non-malignant cervical conditions (1 176 cases), age distribution by histological group*

Histological group (score)	Simple chronic cervicitis (0)	Slight dysplasia (1)	Moderate dysplasia (2)	Overall (0+1+2)
Age				
Median	38 0	37 0	38 0	38 0
Mean ± S.D.	38 6 ± 8 4	38.3 ± 8.7	39 4 ± 8 6	38 6 ± 8.5
Range	11 71	22–68	26–63	11–71
Number of cases	856	239	81	1 176

Analyses of variance on age means: random fluctuations only

Table III. *Epidermoid cervical carcinoma (713 cases) age distribution by histological group*

Histological group (score)	Intra-epithelial (3)	Invasive		
		Early (4)	Late (5)	Overall (4+5)
Age				
Median	39 0	41 0	45.0	43 0
Mean ± S D	39 6 ± 7.5	42.2 ± 7 8	43 6 ± 5.3	43.5 ± 5.6
Range	27–62	25–66	23–80	23–80
Number of cases	64	57	592	649

Analysis of variance on age means.
Between the three groups (scores 3, 4, 5): P < 0.005
Intra-epithelial vs pooled invasive: P < 0.005
Among invasive (early vs late). 0 05 < P < 0.10

evident when the five groups of definite epithelial abnormalities (scores 1 to 5) are studied in advancing order (Tables II and III):

38.3 39 4 39 6 4_2 43 6

These data seem to be consistent with an overall positive association between age at diagnosis and advancing grade of epithelial alterations.

Regression f age at diagnosis on advancing histological score of epithelial alterations

The data were first tested for a possible linear regression of age on advancing, equidistant score of all observed histological conditions of the cervical epithelium (Groups 0–5). This overall analysis disclosed findings consistent with an overall positive but curvilinear relationship

A corresponding analysis was then carried out after the group of no or negligible epithelial abnormalities (Group 0) had been excluded:

Results of Analysis of Varianc on Age Means; Series 1–5 (1 033 ases)

Between epithelial abnormalities (scores 1–5):	P < 0.005
Linear regression of age on advancing score:	P < 0 005
Deviations from linearity	P > 0.25

The deviations from linearity were now attributable to random variation only The findings in series 1 to 5 are therefore consistent with a linear relationship between age at diagnosis and advancing grade of cervical epithelial alterations.

Table I *683 cases of invasive carcinoma of the uterine cervix in Koreans (1958–67) observed age distribution and estimated relative age incidence*

Decade	–29	–39	–49	–59	60–
Observed number of cases	29	218	262	138	36
Percentage of all cases (*a*)	4.2	31 9	38.4	20_	5.3
Total female population estimated mean percentage 1957–65[a] (*b*)	16.3	13 4	9.3	6.3	6.6
Estimated relative age incidence (*a*/*b*)	0.26	2.38	4 13	3._1	0 80

From figures given by Lee et al (1959) and Korea Annual (1967).

culated from data given by Lee Lee & Yun (21) and from *Korea Annual 1967* (16). This population distribution has been compared with the observed distribution by age of cases of invasive cervical carcinoma, in an attempt to estimate the liability of South Korean women to this disease in each age group.

A total of 651 (95.0%) f the invasive cervical cancers were cases of epidermoid carcinoma, and the remaining 34 were adenocarcinomas. The age at diagnosis was not known in two of the former patients, leaving 649 for age studies in the epidermoid cancer series. As many as 592 of these patients suffered from histologically late invasive growths (classified as *Group 5* in this study). The remaining 57 comprised epidermoid cancer cases of superficial infiltration only (early invasi e *Group 4*).

Sixty-fou cases of intra-epithelial cervical carcinoma were diagnosed this series (*G oup 3*) represented patients with severe dysplasia as well as cases of the classical carcinoma-in-situ pattern. Other cases of cervical epithelial abnormalities totalled 320, 81 of which were patients with moderate squamous-cell dysplasia (*Group 2*), the remaining 239 being cases of slight dysplasia (*Gro p 1*). The morphological criteria of dysplasia and intra-epithelial carcinoma conform with those stated by Robbins (32) and Hertig & Gore (9) respectively

Finally 856 cases of simple chronic cervicitis of all grades were included for comparati purposes these w re patients with negligible or no demonstrabl histological alterations in the cervical epithelium, apart from basal-cell hyperplasia in some cases (*Group 0*).

The age of the patient refers to the ge (in years, last birthday) at the time of the histological diagnosis. The ge distributions were compared by means of con entional statistical methods (34). No subdivisions f the six series ha e been made with regard to physiological, social and environmental factors which are known or suspected to be in ol ed in the initiation or promotion f cervical cancer

About one-half of the present series w re in-patients at the NMC, the other half were mainly out-patients and comprised large percentage of rural population cases. It is at present, because of many social and medical difficulties, impossible to do comprehensive studies of morbidity and mortality statistics in Korea (26). The NMC cares, however for more of the rural population than any other large hospital located in the urban area of South Korea. The Center is no charity hospital but treats paying as well as free patients. The cases belong mainly to the lower socio-economic groups. The distribution of the whole Korean population, on the other hand, shows a marked preponderance of the lower social groups.

RESULTS

The results of the age studies are given in Tables I–III and in Fig. 1 All age distributions were unimodal and without any marked skewness.

Invasive cervical carcinoma, overall age distribution and estimated age incidence

The 683 patients of known age with invasive carcinoma were first examined as a whole. Table I shows the observed age distribution and the estimated relative liability to cervical cancer in the female population both distributions reveal a peak within the fifth decade

The observed age at diagnosis ranged from 23 to 80 years the mean was $43\ 6 \pm 5.9$ (S.D), and the median at 43.0 years. Excluding the cases of adenocarcinoma, the overall age of the remaining cases of epidermoid cancer averaged $43.5 \pm 5\ 6$ years the median still being at 43 0 (Table III)

The mean age in patients with adenocarcinoma was $45.5 \pm 10\ 0$ years i.e 2.0 years above that of the epidermoid cancer series. The standard deviation of the latter group is conspicuously below that of the adenocarcinoma cases and the corresponding variances proved heterogeneous ($P < 0\ 005$) implying that the observed age span in patients with cervical adenocarcinoma was significantly larger than in those with epidermoid cancer

Non-malignant conditions (Groups 0 1 and 2 1 176 cases)

Table II shows the observed age distributions for these three series The mean age was 38 6 years in patients with simple chronic cervicitis, 39 4 in those with moderate squamous-cell dysplasia, and 38.3 years within the intermediate Group 1 the latter series of slight dysplasia thus revealing an average age a little below that of simple chronic cervicitis.

46 to 48 years (5 19 39) the median age was 47 years in 3 380 Swedish cases 1958–61 (15)

The mean age at diagnosis was 44 years in the large Indian series of Mali et al. (23), and 48.4 among 81 Korean cases examined from 1925 to 1939 and reported by Yun (41). The age averaged 43.5 years among all cases of invasive cervical carcinoma of the epidermoid type in the current material. The peak of the age distribution curve was also in the fifth decade in the Korean series of Lee, Lee & Kim (20)· there is thus no marked mean age difference between the above Oriental and Occidental series of invasive cervical cancer cases.

Rewell (29) has shown that the mean age for cervical cancer in South India is many years lower than in England. When corrections are made for the far fewer older women in India than in England, however the age of maximum incidence in India is only about nine years lower than in England. It is equally important to realize this point when discussing the relative incidence in the South Korean population, in which around 80% of the women were below the age of 40 years in the 1957–65 period, only about 15% being within the 40–59 age bracket (38) The estimated relative liability to invasive cervical carcinoma in the South Korean population was, however in the fifth decade in our series, and thus in agreement with the findings in the large American series of *Hertig & Gore (9)* The estimated incidence was, moreover higher in the 50–59 age group than in that of 30–39 years in the present Korean series.

Epidermoid cervical carcinoma is rare before the age of 20 years (9 15) The youngest patient was 23 years of age in the current series and 21 years old in the Finnish series of Kahanpaä (12), but four Indian cases seen by Mali et al. (23) occurred in teenagers, the youngest being 18 years old.

From the accumulated evidence, there is now no doubt that invasive epidermoid carcinoma is often preceded for many years by intra-epithelial carcinoma (39). There is no invasive cancer which at one stage was not cancer in situ (23, 3). It is of interest to note that the ethnic distribution of intra-epithelial and invasive cervical neoplasia is similar (9 10).

The currently demonstrable age pattern is an interesting one in relation to the findings of Stern & Dixon (35). In a study concerning the relative importance of eight variables in separating normals from the stages of cancer of the cervix and in discriminating between stages, these authors used multivariate analysis and found that age was the only variable important in separating the pre-invasive from the invasive stage.

In a later study by Stern et al. (36), the relative importance of twenty environmental, social and physiological factors that may be related to the presence or absence of cervical cancer was evaluated in a series of married women The two variables associated with ageing or the passage of time—age of the patients and years since menopause—showed a significant trend proceeding from normals to those with invasive cancer in postmenopausal women, while the age at entry was without demonstrable importance in premenopausal individuals. In comparisons between stages of cancer age, with some additional information contributed by knowledge of race, separated pre-invasive from invasive cancer in the postmenopausal women. The present Korean series has been analyzed without respect to pre- or post-menopausal state we had incomplete information on this and other clinical factors in a number of patients, and the subdivision of the cases has been based on conventional histological criteria only

Nielson et al. (24) give the peak age incidence in cases of intra-epithelial carcinoma in the 30–39 bracket, and the mode of the age distribution was at 40 years in 140 Swedish cases 1921–53 (13) The age range is wide, however, and according to Hertig & Gore (10) from the twenties to the seventies. Recent results of cytological examinations on more than 180 000 American women reveal that carcinoma in situ reached its maximum incidence rate in females aged 25 to 29 years (3) The mean age was, however 34 7 years in the series of Kramer & Kay (18), 37 1 in that of Galvin & TeLinde (5), 38 in that of Hertig & Gore (10), 40.9 in the 1963 report from the Swedish Cancer Registry (31), and 41.6 in a series reported from a Norwegian county screening program (1). The average age in cases of intra-epithelial neoplasia was 39 years in the Indian series of Mali et al. (23) and 39.6 years in the current Korean series; the findings in these two Oriental series are thus rather close and in general agreement with most figures from Western countries.

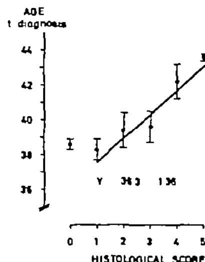


Fig. 1. Six histologically differentiated pathological conditions of the uterine cervix in 1 889 Korean women. With linear regression of age on advancing score of epithelial abnormalities (scores 1–5: 1 033 cases). Mean age at diagnosis ± S.E.

Scores: 0 Simple chronic cervicitis, *1* Slight squamous-cell dysplasia, *2* Moderate dysplasia, *3* Intra-epithelial carcinoma, *4* Early invasive epidermoid carcinoma, *5* Late invasive epidermoid carcinoma.

This linear relationship between age (y) and histological score (x) is shown in Fig. 1 and expressed by:

$$Y = 36.3 + 1.36\,x$$

The 95% confidence interval for the regression coefficient was estimated at 1.36 ± 0.24, i.e. from about 1.1 to 1.6. The step-specific time lag in mean age at diagnosis was, on this level of confidence, thus from approximately 1.1 to 1.6 years for advancing epithelial alterations ranging from slight dysplasia to late invasive epidermoid carcinoma. The mean age difference predicted by the present data was, accordingly, only 4.4 to 6.4 years between the former and the latter condition.

DISCUSSION

The incidence of invasive cervical carcinoma observed in the current Korean series is in accordance with most Oriental figures, while the distribution by type follows a general pattern that seems unrelated to different geographical regions. Epidermoid cervical carcinoma is one of the most important of all cancers in European countries, in most of them second only to breast cancer in frequency, but this is reversed in Asiatic countries. The frequency is, however, far greater in Indians and Chinese than in the Malays, although the latter people have a high birth rate (29).

In Korea, Lee, Lee & Yun (21) found a total of 310 cases of cervical carcinoma among 2 705 consecutive cases of histologically examined malignant tumours from major hospitals between 1956 and 1959. This tumour constituted the most frequently encountered primary malignant neoplasm in females, and was responsible for 30.07% of all malignant disease in this sex. In a later communication by Lee, Lee & Kim (20), an additional 235 surgical and autopsy cases provided an observed total of 545 cervical carcinomas, that constituted about the same fraction (30.13%) of all cancers in Korean women. Carcinoma of the uterine cervix was responsible for no less than 65.6% of all primary genital cancer in females within the current Korean series, and for 37.6% of all malignant disease of known primary site in this sex.

These levels are much higher than those generally reported in Western series. Figures from Sweden show that cancer of the uterine cervix accounts for about 35% of the gynaecological and for only around 8% of all female cancer in that country (15). Grimstvedt & Waaler (6) have reviewed an autopsy material comprising 1 976 cases of malignant disease in Western Norway 1913–54 and concluded that carcinoma of the female sex organs (including breast) was responsible for one-third of the malignant neoplasms in females. The corresponding fraction was 55.4% of a total of 1 885 malignant tumours found in the present series of Korean women.

Regarding adenocarcinoma, no particular mention is found in the literature of racial or ethnic differences in the relative incidence of this type of cervical malignancy. The estimated frequency of cervical adenocarcinoma ranges in different series from 1.6% to 11.7% of cervical cancers, with an average of 5.7% (39). The frequency was 5.0% in our material, and thus in close agreement with the general figures.

The age distribution of women with uterine malignancy is of special significance in indicating the age group that should be surveyed for cancer (23). The mean age in Western series of invasive cervical carcinoma ranges from 39.8 years in the American material of Kramer & Kay (18) to 51.4 in the Swedish of Ringertz et al. (31). Reports based on most larger series give averages from

39 years and that of invasive cancer 44 years. This age distribution by histological stage is quite similar to that observed in the present Korean series.

The gradual rise in average age at each successive stage from carcinoma in situ to invasive carcinoma was probably not significant in the American series of Hertig & Gore (9). The present Korean material revealed a similar pattern, with the sole exception of the established difference in mean age between intra-epithelial and early invasive epidermoid cancer. The upward trend in average age was, however, significant in both series, and in the current one consistent with a linear type of regression of age at diagnosis on advancing score of epithelial alterations. The mean age in cases of simple chronic cervicitis was somewhat higher than in the group of slight dysplasia, this might be caused by a presumably high number of patients suffering from simple chronic cervicitis and never developing dysplastic lesions.

The findings of Stern et al. (36), as well as the increasing trend in mean age at advancing stages of epithelial alterations observed by other authors and in the present Korean series, support the concept of a step-wise process in the evolution of epidermoid cervical carcinoma.

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Most recent authors find that the age distribution curve of intra-epithelial carcinoma shows a peak that occurs from 8 to 12 years earlier than that of invasive cancer in general close to 10 years (3 9 13 15 24 31) The observed mean age in cases of intra-epithelial carcinoma in the present Korean series was only 3.9 years below that of all invasive epidermoid cancers the 95 confidence interval estimate for the true difference in mean age ranging from 2 8 to 5 years. The observed difference amounted to 5 years in the Indian material of Mali et al. (23) the mean age discrepancy thus being of the same order in these Oriental series, and conspicuously below that of most Western reports.

Swedish follow up studies reported by Kottmeier (17) and Karlstedt (13) are particularly interesting in this connection Thirty-one cases of carcinoma in situ diagnosed in curettings or small biopsies were kept under observation and an invasive carcinoma developed in 22 of these patients from 8 months to 19 years after the initial diagnosis was made (13 17) Kottmeier (17) also reports a series of 163 patients with carcinoma in situ treated conservatively (i.e. not subject to hysterectomy or radiotherapy) and an invasive carcinoma developed in 14 of these 163 cases after an interval varying from only 2 months to 8 years

The significance of squamous-cell dysplasia in epidermoid cervical carcinogenesis has received considerable attention in recent years. There is now evidence of a chronological progression from mild dysplasia through moderate and severe dysplasia to carcinoma in situ and invasive carcinoma (30) Kramer & Kay (18) find that there has been adequate documentation of such a stepwise progression and the present age pattern supports this theory of evolution The incidence rates for dysplasia rose rapidly and at an earlier age than those of carcinoma in situ in the report of Dunn & Martin (3) Dysplasia remained at a high incidence over a longer age span than carcinoma in situ but decreased precipitously after the age of 40 the latter lesion continuing at a low level after the age of 35 years

Willis (39) states that there is no clear distinction between true carcinoma in situ and carcinoma-like dysplasia, and the group of intra-epithelial neoplasia in the present study includes cases of severe squamous-cell dysplasia as well as those of the classical in-situ pattern. We found justification for this also in the work of Kirkland et al (14) who found that some cases of severe dysplasia exhibited mitotic and/or chromosomal abnormalities similar to those of invasive carcinoma, and suggested that these lesions are as dangerous as the classical variety of carcinoma in situ.

Stern et al (36) reported on a series of 904 randomly selected controls, and cases of dysplasia, pre invasive and invasive squamous-cell carcinoma in married women The four groups were compared in several ways, and also analysed by an arbitrary scoring system which assumed that there was a progression from one group to the other in the above order This arrangement has been adopted in the current Korean series. Stern et al. (36) stressed that this arrangement in particular placed the individuals with dysplasia and those with pre invasive cancer as intermediate between normals and invasive cancer and found some evidence for this assumption in the report of Stern & Neely (37) in which dysplasia showed regression to normal, recurrence, and progression to cancer

Several authors have reported an upward trend in mean age at successive stages of cervical epithelial alterations. The average age of patients with carcinoma in situ was 38 years in the American series of Hertig & Gore (9) 4_2 years among those with doubtful early stromal invasion, 48.5 in cases with clinically unsuspected carcinoma, and 51 years in those with invasive cancers of the clinical stages I to IV In the series of Kramer & Kay (18) premalignant anaplasia occurred at an average age of 30 1 years, carcinoma in situ at 34 7 years and invasive squamous-cell carcinoma at 39 8 years

Among mass screening cases examined in the Norwegian Radium Hospital 1959–65 the average was 37.3 years in patients with advanced dysplasia, 41 6 in cases of carcinoma in situ, 4_2 in those of early invasive and 45.5 years in patients with late invasive cancer (1)

Mali et al (23) compared the age incidence of advancing grades of cervical epithelial alterations diagnosed in 44 919 vaginal smears from Indian women, and found a definite increase The average age of cervical dysplasia grade I was 34 years for grade II 35 years and for grade III 37 years while that of intra-epithelial carcinoma averaged

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THE EFFECT OF ANTIBIOTIC THERAPY ON MYCOPLASMA IN THE FEMALE GENITAL TRACT

In vitro and in vivo Studies on the Sensitivity of Mycoplasma hominis and T-Mycoplasmas to Tetracyclines and other Antibiotics

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Abstract. The susceptibility of M. hominis and T-mycoplasmas to different antibiotics in vitro has been studied. A clinical assessment of metacycline, lincomycin and chloramphenicol in genital infections in the female, with special reference to M. hominis and T-mycoplasmas is also presented. After treatment with metacycline or lincomycin, M. hominis was rarely isolated and in the majority of cases such therapy was followed by disappearance of symptoms and signs of infection and rapid re-establishment of vaginal flora dominated by Doderlein' bacilli. In patients who had received chloramphenicol, cultures yielded in most cases growth of M. hominis, and signs of infection persisted after treatment. The effect of treatment with metacycline, lincomycin or chloramphenicol on M. hominis correlated well with the susceptibility of this organism found in vitro. Cultures for T-mycoplasmas showed that these organisms could be recovered in about the same frequency before and after therapy.

The role of Mycoplasma in infections of the female genital tract has been the subject of many investigations since the first report in 1937 by Dienes & Edsall on the isolation of a strain of Mycoplasma from an abscess of a Bartholin's gland. Mycoplasma hominis may often be isolated from females with signs of genital infections but rarely from healthy women (5), while T-mycoplasmas occur with a similar frequency in women with genital infections and healthy females (13). Occasionally also Mycoplasma fermentans may be isolated from the lower female genital tract (3).

Reports have been published on the isolation of M. hominis from the Fallopian tubes of patients with acute salpingitis (12), from the blood of women with post-partum endometritis (17, 19, 21) and from foetuses in cases of spontaneous abortion (8, 10, 11).

Mycoplasma organisms lack the cell-wall structure characteristic of bacteria and are consequently not sensitive to penicillin and other antimicrobials that interfere with the biosynthesis of the bacterial cell wall. On the other hand, they are generally sensitive to antibiotics interfering with protein synthesis in the bacterial cell.

The present study concerns the susceptibility of M. hominis and T-mycoplasmas to antibiotics in vitro. It also deals with the effect of treatment with metacycline, lincomycin and chloramphenicol on M. hominis and T-mycoplasmas in women with infections of the lower genital tract.

MATERIAL AND METHODS

In vitro studies

I. *Mycoplasma hominis*

Organisms: The method of isolation and identification of M. hominis has been described earlier (12). The strains examined were isolated from the cervix or the urethra of females with signs of genital infections. The number of strains of M. hominis tested appears in Table I. Broth cultures of the strains were stored at −70°C until used.

Determination of the minimum inhibitory concentration (MIC) by the agar-plate dilution technique

Antibiotics: Tetracycline hydrochloride (Pfizer), oxytetracycline hydrochloride (Pfizer), metacycline hydrochloride (Roerig), doxycycline hydrochloride (Pfizer), lincomycin

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Table I. *In vitro susceptibility of M. hominis to various tetracyclines, lincomycin, chloramphenicol and erythromycin*

Antibiotic	No. of *strains*	MIC (µg per ml) Cumulative number and percentage (within brackets) of strains inhibited by: 0.05	0.1	0.2	0.4	0.8	1.6	2.0	4.0	8.0	16.0	100
Doxycycline	58	0 (0)	24 (41)	54 (90)	58 (100)							
Tetracycline	25		0 (0)	5 (20)	23 (92)	25 (100)						
Metacycline	58		0 (0)	9 (16)	43 (74)	58 (100)						
Lincomycin	24			0 (0)	1 (4)	4 (17)	24 (100)					
Oxytetracycline	58						0 (0)	14 (24)	56 (97)	58 (100)		
Chloramphenicol	38							0 (0)	4 (11)	28 (74)	38 (100)	
Erythromycin	40											0 (0)

RESULTS

In vitro Studies

M. hominis

The results of the sensitivity tests with M. hominis are presented in Table I. The sensitivity pattern of the different strains of M. hominis was very similar. Among the different strains of M. hominis, a variation of the MIC of a given antibiotic of more than two dilution steps was only exceptionally found (Table I).

The activity of the drugs tested was uniform over a wide range of inocula. The sensitivity of five strains of M. hominis to doxycycline was tested by making tenfold dilutions of the inocula. There was no change of the MIC when the inocula contained from 10^7 to 10 CFU per ml. When the inocula contained 10^9 CFU per ml the MIC was changed from 0.1 to 0.05 µg per ml. Analogous results were obtained with metacycline, tetracycline and chloramphenicol.

In order to obtain a measure of the reproducibility of the agar-plate dilution technique, the sensitivity of three strains of M. hominis to the antibiotics tested was assayed on five different occasions. With the same strain and a certain antibiotic, a variation of the MIC of more than plus or minus one step of dilution of the antibiotic was not observed from one test occasion to another, providing that the plates were read after the same incubation time.

Incubation of the plates aerobically instead of in an atmosphere of nitrogen and carbon dioxide had no influence on the results of the sensitivity tests, nor had the omission of thallium acetate from the sensitivity test medium.

T-mycoplasmas

The results of the sensitivity tests of T-mycoplasmas are tabulated in Table II. The sensitivity of T-mycoplasmas to the four tetracycline drugs and to chloramphenicol was similar to that of M. hominis. In contrast to M. hominis, T-mycoplasmas were sensitive to low concentrations of erythromycin. They also differed from M. hominis in being resistant to high concentrations of lincomycin.

Between different strains of T-mycoplasmas, a variation of the MIC of a given antibiotic of more than two dilution steps was not observed (Table II).

Table II. *In vitro susceptibility of T-mycoplasmas to various tetracyclines, erythromycin, chloramphenicol and lincomycin*

Antibiotic	No. of strains	Cumulative number of strains inhibited by MIC (µg per ml) 0.025	0.05	0.1	0.2	0.4	0.8
Doxycycline	14	0	3	10	14		
Metacycline	10		0	2	8	10	
Tetracycline	8			0	3	7	8
Oxytetracycline	10				0	4	10
		MIC 0.15	0.3	0.6	1.2		
Erythromycin	13	0	7	1	13		
		MIC 4.0	8.0	16.0	32.0		
Chloramphenicol	12	0	4	10	12		
		MIC 10.0	20.0	40.0	80.0		
Lincomycin	9	0	4	7	9		

(Leprolin), chloramphenicol (Erco) and erythromycin lactobionate (Abbott). Stock solutions of the antibiotics were prepared with sterile water. The final concentration in µg per ml of the antibiotic was based upon the active portion of the drug.

Medium. Heart-infusion broth (Difco) 100 ml, horse serum 20 ml, yeast extract (baker's yeast 25 %) 10 ml, thallium acetate 1:2 000 (final concentration), Ionagar No. 2 (Oxoid) 1.1 %. Two-fold dilutions of the stock solutions of the antibiotics were made in the medium. 20 ml of the drug-medium mixture was poured into Petri dishes, 9 cm in diameter.

Inoculum. The inoculum contained 10^{4} to 10^{5} viable organisms per ml measured as colour forming units (CFU). A 0.1 ml portion of the broth cultures was streaked over a quarter of each plate in the series containing the antibiotics.

Controls. Inoculations were made on plates containing distilled water instead of antibiotic solutions.

Incubation. The plates were incubated at [illegible] °C in an atmosphere of 90 % nitrogen and 10 % carbon dioxide and read under a microscope after 48 hours of incubation.

Reading of the tests. The MIC was taken to be the least amount of antibiotic causing no visible growth as judged by microscopic examination of the plates. The tests were accepted only if confluent growth had developed on the control plates.

II. T-mycoplasmas

Organisms. The procedure of isolation and identification of T-mycoplasmas has been described earlier (13). The strains under investigation were isolated from the cervix or the urethra of women with genital infections. The number of strains examined is presented in Table II. Broth cultures of the strains were stored at −80 C until used.

Sensitivity tests

Antibiotics. The antibiotics were those used in the sensitivity tests on M. hominis by the agar plate dilution technique. Stock solutions of the antibiotics were made in sterile distilled water the day the tests were made.

Medium. Trypticase soy broth (Baltimore Biol. Lab.) 3 %, horse serum 20 %, urea 0.04 %, and phenol red 0.0[illegible]. The pH of the medium was 6.0.

Performance of the test. The metabolic-inhibition technique, described by Purcell et al. (15) was adapted for the estimation of antibiotic sensitivity. The tests were made with a micro-titration system. Serial two-fold dilutions of the stock solutions of the antibiotics were made in 0.1 ml of the sensitivity test medium. One volume (0.025 ml) of a 16-hour-old broth culture of the strain to be tested was then added to each cup. The trays were sealed with cellulose covers, carefully shaken and incubated at 3[illegible] C. The tests were run in duplicate.

Controls. As controls, antibiotic-free cultures and uninoculated culture medium were included.

Reading of the tests. The tests were read when the pH of the antibiotic free cultures had changed approximately 0.8 pH units (determined by a comparison of the colour of the medium with the colour of an uninoculated medium including phenol red with known pH values). The MIC was taken to be the least amount of antibiotic that inhibited growth as judged by the absence of colour change of the medium.

Clinical Study

The case material included [illegible] non-pregnant, non-menstrual women of childbearing age with symptoms and signs of infection of the genital tract, and in whom cultures from cervical specimens had yielded growth of M. hominis. None of the women had received antibiotic treatment prior to the examination.

Diagnostic criteria of infection of the lower genital tract. All the [illegible] investigated women complained of vaginal discharge and 43 of them had additional symptoms, such as lower abdominal pain, burning, pruritus or pain in the vulva or the vagina. Examination revealed pathological vaginal secretion and diffuse or patchy reddening of the vaginal epithelium. The vaginal secretion was deemed pathological when its colour was yellowish, greyish or greenish and its amount was more than normal or when it was malodorous.

Sampling and cultural procedures. Specimens collected from the urethra and the cervix were cultured for Mycoplasma, Neisseria gonorrhoeae and Trichomonas vaginalis. The sampling and cultural techniques were those described earlier (1[illegible]). Smears of vaginal discharge were fixed in ethanol and stained according to Papanicolaou.

Treatment. Twenty-two of the patients were treated with 600 mg of metacycline (Rondomycin®) orally daily for 8 days and 9 of the women received 1 500 mg of lincomycin (Lincocin®) daily for 8 days. To [illegible] of the cases 1 000 mg of chloramphenicol (Kloramfenikol®) in combination with 1 200 mg of phenoxymethylpenicillin (Calcipen®) was administered orally daily for 10 to 14 days. In most cases [illegible] (Pimafucin®) was administered locally into the vagina during the period of the antibiotic therapy. To [illegible] patients in whom T. vaginalis was demonstrated 600 mg of metronidazole (Flagyl®) given orally for 1 week was added to the [illegible] treatment.

Follow-up. Examinations and control cultures were made within 1 week after therapy was completed, and in cases recovered for a period of 4 months. The women were instructed not to have sexual intercourse until the first control culture had been made.

Criteria for recovery after treatment. These were complete relief of symptoms, normal conditions at examination, i.e. sparse, colourless vaginal contents, pink vaginal epithelium, normal secretion from the cervical canal or absence of pain on bimanual palpation of the uterus and adnexa, and vaginal smears in which the bacterial flora was dominated by Döderlein bacilli.

Table I. *In vitro susceptibility of M. hominis to various tetracyclines, lincomycin, chloramphenicol and erythromycin*

Antibiotic	No. of strains	MIC (µg per ml) Cumulative number and percentage (within brackets) of strains inhibited by: 0.05	0.1	0.2	0.4	0.8	1.6	2.0	4.0	8.0	16.0	100
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Tetracycline	25		0 (0)	5 (20)	23 (92)	25 (100)						
Metacycline	58		0 (0)	9 (16)	43 (74)	58 (100)						
Lincomycin	24			0 (0)	1 (4)	4 (17)	24 (100)					
Oxytetracycline	58						0 (0)	14 (24)	56 (97)	58 (100)		
Chloramphenicol	38							0 (0)	4 (11)	28 (74)	38 (100)	
Erythromycin	40											0 (0)

RESULTS

In vitro Studies

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The activity of the drugs tested was uniform over a wide range of inocula. The sensitivity of five strains of M. hominis to doxycycline was tested by making tenfold dilutions of the inocula. There was no change of the MIC when the inocula contained from 10^5 to 10 CFU per ml. When the inocula contained 10^2 CFU per ml, the MIC was changed from 0.1 to 0.05 µg per ml. Analogous results were obtained with metacycline, tetracycline and chloramphenicol.

In order to obtain a measure of the reproducibility of the agar-plate dilution technique, the sensitivity of three strains of M. hominis to the antibiotics tested was assayed on five different occasions. With the same strain and a certain antibiotic, variation of the MIC of more than plus or minus one step of dilution of the antibiotic was not observed *from one test occasion* to another providing that the plates were read after the same incubation time.

Incubation *of the plates aerobically instead of* in an atmosphere of nitrogen and carbon dioxide had no influence on the results of the sensitivity tests, nor had the *omission of* thallium acetate from the sensitivity test medium.

T-mycoplasmas

The results of the sensitivity tests of T-mycoplasmas are tabulated in Table II. The sensitivity of T-mycoplasmas to the four tetracycline drugs and to chloramphenicol was similar to that of M. hominis. In contrast to M. hominis, T-mycoplasmas were sensitive to low concentrations of erythromycin. They also differed from M. hominis in being resistant to high concentrations of lincomycin.

Between different strains of T-mycoplasmas, variation of the MIC of a given antibiotic of more than two dilution steps was not observed (Table II).

Table II. *In vitro susceptibility of T-mycoplasmas to various tetracyclines, erythromycin, chloramphenicol and lincomycin*

Antibiotic	No of strains	Cumulative number of strains inhibited by MIC (µg per ml) 0.025	0.05	0.1	0.2	0.4	0.8
Doxycycline	14	0	3	10	14		
Metacycline	10		0		8	10	
Tetracycline	8			0	3	7	8
Oxytetracycline	10				0	4	10
		MIC 0.15	0.3	0.6	1.2		
Erythromycin	13	0	7	12	13		
		MIC 4.0	8.0	16.0	32.0		
Chloramphenicol	12	0	4	10	12		
		MIC 10.0	20.0	40.0	80.0		
Lincomycin	9	0	4	7	9		

Table III *Isolation rate of M hominis and T-mycoplasmas in patients treated with metacycline lincomycin or chloramphenicol in combination with penicillin*

Antibiotic	No of cases	M hominis isolated before therapy	M hominis isolated after therapy	T mycoplasmas isolated before therapy	T mycoplasmas isolated after therapy
Metacycline	22	22	2	15	11
Lincomycin	9	9	1	4	4
Chloramphenicol and penicillin	24	24	22	16	9

To check the reproducibility of the method used for the sensitivity test with T-mycoplasmas, two strains were tested on 10 different occasions to doxycycline erythromycin and chloramphenicol From one test occasion to another a variation of the MIC exceeding two steps of dilution of the antibiotics was not observed

Clinical Study

The isolation rate of M. hominis from the lower genital tract after therapy is presented in Table III In the 22 women treated with metacycline the organism was cultivated from two after treatment. The corresponding figure for the nine women who were treated with lincomycin was one In the 24 patients who were treated with chloramphenicol in combination with penicillin the isolation rate of M hominis was almost the same before as after the treatment (Table III)

Repeated cultures showed that M hominis persisted in the lower genital tract for at least 4 months in those cases where the treatment failed to eradicate the organism. In those women in whom the first culture after therapy yielded no growth of M hominis, the organism could not be recovered on any of the following cultures for up to 4 months.

As appears from Table III, T mycoplasmas were frequently recovered after therapy with metacycline, lincomycin, or chloramphenicol and penicillin.

Cytological examinations of vaginal smears collected before the treatment revealed signs of an infective process in all the cases, and in no instance was there a vaginal flora dominated by bacteria morphologically of Döderlein's type. Smears collected after therapy from 19 of the 22 women treated with metacycline showed a vaginal bacterial flora dominated by Döderlein's bacillus whereas the corresponding figure for those who had received chloramphenicol and penicillin was 4 out of 24

Neisseria gonorrhoeae was isolated before, but not after therapy from 4 of the women who were treated with metacycline and from 5 of those who were treated with chloramphenicol and penicillin From the latter 5 patients, M hominis was still isolated after therapy

T vaginalis was demonstrated in 14 of the investigated women Treatment with metronidazole eradicated the flagellate in all cases. More than 10 days of therapy was, however necessary in one patient.

The result of clinical treatment and its relation to the occurrence of M hominis is shown in Table IV Of the 31 cases with clinical cure, 5 still harboured M hominis after treatment. This was in contrast to the 24 cases with persisting symptoms and signs, 20 of which still harboured the organism after therapy The difference in the isolation rate of M hominis after therapy in patients who fulfilled the criteria for cure and those who did not, was significant ($\chi^2 = 21\ 175$ $p < 0\ 001$) Clinical recovery plus eradication of M. hominis was found in 91 67 and 8% of the women treated with metacycline, lincomycin or chloramphenicol in combination with penicillin respectively

Table IV *The effect of therapy on M hominis and on symptoms and signs of genital infection*

Antibiotic	M hominis after therapy	No of cases	Criteria for clinical recovery fulfilled[a]	Persisting symptoms and/or signs of infection
Metacycline	Isolated	2	0	2
	Not isolated	20	18	
Lincomycin	Isolated	1	0	1
	Not isolated	8	6[b]	2
Chloramphenicol and penicillin	Isolated	22	5[c]	17
	Not isolated	2	2	0

[a] See text.
[b] Cytological examinations of vaginal smears not made
[c] Cytological examinations of vaginal smears not made in three of the cases.

DISCUSSION

Many researchers have reported a considerably higher isolation rate of M. hominis in women with signs of infection in the genital tract than in healthy females (6). However there has been no unanimity as to the frequency in which M. hominis occurs in "healthy" women. This may depend on the fact that different criteria have been used for the definition of "health in this respect. In women without signs of genital infection in the childbearing age, the vaginal flora is generally dominated by Döderlein's bacillus, and Freundt (5) has shown that such women rarely harbour M. hominis in the lower genital tract. Using the criteria for clinical recovery already mentioned as definition of health, M. hominis was, in an earlier investigation, isolated from the lower genital tract in only 4% of a group of healthy females. The corresponding figure in a group of women with signs of genital infections was 54% (12). Hayflick & Chanock (9) stressed the importance of matching groups of infected cases and healthy controls as to age, parity socio-economic status, and to the extent of sexual promiscuity for the evaluation of the role of Mycoplasma in genital infections. Such a matching had been made in our earlier investigation mentioned above The incidence of genital infections in various age-groups in a female population and the distribution of indirect haemagglutination antibody to M hominis were found to be closely correlated (14).

In conformity with earlier reports (2, 20), the present study showed that M. hominis was sensitive to antibiotics of the tetracycline group in vitro. It also showed that the newest member of this group, doxycycline, was the most active one of the tetracycline drugs in vitro.

Among Mycoplasma species isolated from man, M hominis, M fermentans, M. orale and M. salivarium have been shown to be resistant to high concentrations of erythromycin in vitro (18 20), whereas M. pneumoniae and different serotypes of T-mycoplasmas are sensitive to low concentrations of this antibiotic (16). As to T-mycoplasmas and erythromycin, the results of the present study were in agreement with those of Shepard et al. (16) The present study also demonstrated that the in vitro susceptibility of M. hominis to lincomycin differed considerably from that of T mycoplasmas. Whether the marked difference in the sensitivity to erythromycin and lincomycin among different species of Mycoplasma reflects a basic difference between various species is not yet known.

The effects of metacycline, lincomycin and chloramphenicol on M. hominis in vivo corresponded well with the in vitro sensitivity of M. hominis to these antibiotics. In women harbouring M. hominis, treatment with metacycline or lincomycin was followed by eradication of M hominis in 91 and 67% of the cases respectively whereas the corresponding figure for the women treated with chloramphenicol plus penicillin was 8%. In those women in whom M. hominis was isolated after treatment, the criteria for clinical recovery were only rarely fulfilled. Treatment with metacycline was in all but 2 cases followed by a disappearance of symptoms and signs of infection in the lower genital tract and a rapid re-establishment of a vaginal flora dominated by Döderlein's bacillus. Although the occurrence of M. hominis was well correlated with the presence of clinical signs of infection, it was not possible to attribute its presence to any characteristic clinical picture.

The two women who still harboured M. hominis after treatment with metacycline were the only ones among the women investigated who admitted sexual intercourse before control cultures had been made. These women reported that the symptoms disappeared after treatment, but that vaginal discharge recurred after coitus. A reinfection must be suspected in these 2 cases. This suggests that M. hominis may be transmitted by sexual intercourse.

Twenty-three women in the present study had received local vaginal treatment with sulphonamides, metronidazole, methylquinoline, pimaricin and various acidifying drugs prior to the oral antibiotic treatment. From all of these patients, M. hominis was recovered from the lower genital tract after such treatment. Although some women reported relief of symptoms after the local treatment, all complained of persistent discharge, and all had signs of infection. The observation that treatment with acidifying agents was not sufficient to eradicate M. hominis from the lower genital tract is of interest, as it is known that M. hominis in vitro does not thrive in an acid milieu and that the organism seldom may be recovered from the genital tract of healthy women with low vaginal pH (5).

N. gonorrhoeae was isolated in 5 women before but not after treatment with chloramphenicol and penicillin. From all of these cases, M. hominis was isolated after therapy. These women all had signs of infection after the treatment. A corresponding correlation between the occurrence of M. hominis and clinical signs of infection was observed in 12 patients in whom T. vaginalis had been demonstrated before but not after treatment with metronidazole. Thus, in spite of eradication of N. gonorrhoeae and T. vaginalis, signs of infection persisted in those patients from whom M. hominis still could be recovered.

Most studies on T-mycoplasmas have concerned their role in non-gonococcal urethritis in the male. Early investigations suggested a causative role of T-mycoplasmas in this condition, whereas recent studies do not support such a relationship (1, 4, 7).

The hormonal status of the individual seems to be an important factor for the occurrence of T-mycoplasmas in the lower genital tract of the female (13). In an earlier study, T-mycoplasmas were recovered from the genital tract of healthy women in the childbearing age in about half of the examined cases, but rarely from healthy girls or post-menopausal women. In the childbearing age no significant difference was demonstrated in the isolation rate of T-mycoplasmas in healthy women and women with signs of infection of the lower genital tract (13).

With the exception of lincomycin and erythromycin, T-mycoplasmas had a sensitivity pattern similar to that of M. hominis. However the recovery rate of T-mycoplasmas after treatment with metacycline and chloramphenicol differed appreciably from that of M. hominis. Thus, after treatment, T-mycoplasmas were still often recovered from the lower genital tract. An explanation of this "therapeutic failure" might be that T-mycoplasmas located intracellularly in epithelial cells may have escaped the action of the antibiotics. No correlation could be demonstrated between the occurrence of T-mycoplasmas and signs of infection in the lower genital tract.

Side effects from the antibiotic therapy were reported by one woman treated with metacycline and by three women treated with lincomycin. These women complained of diarrhoea. Clinical signs of a superinfection with Candida albicans after therapy was found at the control examination in 7 women, two of whom had been treated with metacycline, one with lincomycin and 4 with chloramphenicol plus penicillin.

The role of M. hominis, as well as other microorganisms, in infections of the lower genital tract of the female has been difficult to evaluate, because in these conditions many different microorganisms occur simultaneously. However, the results of this study seem to indicate that M. hominis plays a role in infections of the lower genital tract of the female. The present study also indicates that tetracyclines would be the antibiotics of choice when M. hominis infections are suspected in the female genital tract.

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Announcements

German Society of Endocrinology

Marius Tausk Award

Submission of manuscripts for the 1972 Marius Tausk Award of DM 15 000,— sponsored by Organon G.m.b.H., München.

Applicants residing in Europe and less than 30 years of age are invited to submit manuscripts of previously unpublished work in the field of clinical and clinical-experimental endocrinology (with the exception of diabetes mellitus) to the President of the German Society of Endocrinology for 1971/72, Prof. Dr H. L. Krüskemper 3 Hannover Podbielskistraße 380, Abt. fur Klinische Endokrinologie, Dept. Innere Medizin der Medizinischen Hochschule. The manuscripts may be in either German or English and should be submitted in two copies not later than October 15th 1971. After receipt of the manuscript has been acknowledged the author is free to have his work published by any journal. The statutes for the Marius Tausk Career Development Award may be obtained from the President of the Society. The Award will be presented at the 18th Symposium of the German Society of Endocrinology in Hannover 1972.

German Society of Endocrinology

Schoeller Junkmann Award

Submission of manuscripts for the 1972 Schoeller Junkmann Award of DM 15 000,— sponsored by Schering AG Berlin an Bergkamen.

Applicants residing in Europe who are less than 40 years of age are invited to submit original manuscripts of previously unpublished work in all fields of endocrinology (with the exception of diabetes mellitus) to the President of the German Society of Endocrinology for 1971/72, Prof. Dr H. L. Krüskemper 3 Hannover Podbielskistraße 380 Abt. fur Klinische Endokrinologie *Dept. Innere Medizin der Medizinischen Hochschule.* The manuscripts may be in either German or English and should be submitted in two copies not later than October 15th 1971. After receipt of the manuscript has been acknowledged the author is free to have his work published by any journal. The statutes for the Schoeller Junkmann Award may be obtained from the President of the Society. The Award will be presented at the 18th Symposium of the German Society of Endocrinology in Hannover 1972.

Acta Obstet Gynec Scand 50 33–36 1971

THE VIENNA METHOD OF PROPHYLAXIS AGAINST COMPLICATIONS AFTER RADICAL SURGERY FOR CANCER OF THE UTERUS[1]

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Abstract. With the help of the Vienna method, the most important urological, intestinal and thromboembolic complications can be reduced substantially: ureterovaginal fistulae from 5 to 0.68%, residual urine from 19.28 to 3.75%, pyelitis and pyelonephritis from 9.28 to 2.73%. Serious postoperative surgical complications, e.g. ileus and postoperative meteorism, can be avoided altogether. Thromboembolic complications dropped from 10.6% (without prophylactic Marcoumar medication) to 2% (with prophylactic Marcoumar medication).

The principal disadvantage of extended radical abdominal surgery for carcinoma of the uterus as against other surgical techniques is that it is associated with higher incidence of urological complications. For instance, ureterovaginal fistulae have been reported in 10 to 14% of cases (Latzko & Schiffmann, 1919; Meigs, 1965; Brunschwig & Frick, 1956; Medina et al., 1960; etc.). Even in recent years their incidence has continued to be high with reported incidences ranging from 4 to 10% (Geller & Hilfrich, 1962; Helo, 1965; Bielfeld & Schneider, 1967; Kelso & Funnell, 1967; Abelli et al., 1968; etc.).

METHOD AND MATERIAL

At the occasion of the Third FIGO Congress in Vienna in 1961 first reported on method to reduce substantially early postoperative urological complications. This method requires drug-induced increase in the activity of the ureteric and bladder muscles (Gitsch & Brandstetter). By leaving the so-called meso-ureter in place and thus preserving the longitudinal ureteric vessels when performing an original Wertheim operation with removal of lymph nodes, Janisch & Palmrich succeeded in reducing the incidence of ureterovaginal fistulae to 1.01% in 520 Wertheim operations performed at the First Department of Obstetrics and Gynecology, University of Vienna Medical School, between January 1 1959 and July 1 1968. Owing to the administration of antibiotics and to suction drainage of the operative field, urological morbidity (primarily persistent hydronephroses) during the period from January 1, 1962 to May 1 1968 decreased from 5% prior to 1962 to 1.4%, whilst mortality directly due to urological complications dropped from 1.36 to 0.30%.

The prophylactic method currently employed at the Departments of Obstetrics and Gynecology, University of Vienna Medical School, is primarily designed to prevent postoperative urological and intestinal complications as well as to reduce the incidence of thromboembolism:

I. Preoperative urological assessment including radio-isotope renogram.

II. Surgical measures

(a) Preservation of the vascularized ureteric sheath (adventitia, paraureter) and formation at the ureterovesical junction of small tissue cone with its base at the bladder wall. In extended radical operations the preservation of tissue bridge between the ureter and the pelvic wall is impossible.

(b) Suction drainage through the vagina. Before placing the external peritoneal suture, provisions are made for active and passive drainage of the dead space created by surgery: small tubes ("Redon") are inserted in each pararectal fossa. For better anchorage the ends of the tubes are shaped to form ring. The tubes pass through the vagina into graded suction bottle suspended at the bedside. The drains which are kept in place by chromic catgut sutures are removed as soon as daily secretions drop below 10 ml. In addition, so-called overflow drain of elastic soft rubber with diameter of 6 to 10 mm and wall thickness of 2 mm is inserted in the vagina. This drain is kept in place by two lateral rectangular flaps arranged at opposite sides.

(c) Optimum surgical technique and haemostasis.

[1] Presented in part at the Sixth World Congress of Gynecology and Obstetrics, New York, April 12–18, 1970.

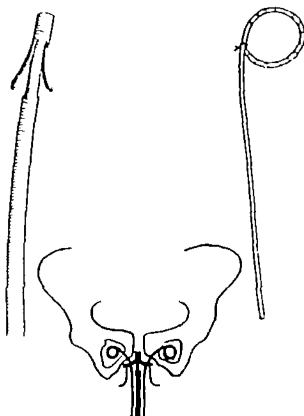


Fig. 1

III. Postoperative prophylaxis

(*a*) A Foley catheter is inserted into the urinary bladder at the beginning of the operation. Drainage is continued until the 20th postoperative day.

(*b*) Antibiotic protection from day 1 to 11 postoperatively (Achromycin). Beginning with the 11th postoperative day a depot sulphonamide is given for 6 weeks and the urine is examined for bacteria every 3rd day until discharge.

(*c*) Drug-induced stimulation of ureteric activity. On the second postoperative day Ubretid (hexamethylene-bis-*N*-methyl-carbaminic acid-3-pyridilester-bromomethylate produced by Österreichische Stickstoffwerke AG, Linz/Danube, Austria), a long-acting cholinesterase inhibitor 0.1 mg per 10 kg body weight, is injected i.m. In addition, Travelin (a drug similar to Dramamine), 150 mg i.m., is given to counteract nausea which occurs occasionally. For Travelin injections a separate syringe should be used. Injections are given every third day and continued to the 77th postoperative day. One day prior to Ubretid-Travelin injections, 1.0 mg Mestinon (Hoffmann La Roche AG, Basle, Switzerland, pyridostigmine) is administered. (For details, see literature available from E. Gitsch). Since July 1, 1968 at First Dept. of Obstetrics and Gynaecology Ubretid has been administered subcutaneously not intramuscularly. This policy has been adopted to avoid haematoma formation, since patients have simultaneous medication with Marcoumar as a prophylactic measure against thrombosis. Experience has shown that higher doses of Ubretid are required when it is administered subcutaneously. Therefore, two-thirds of the initial dose are given on the day following the first injection. This subcutaneous dose produces essentially the same results as the above i.m. dosage.

(*d*) As sporadic cases of intolerance reactions have been observed with Ubretid and administration had to be discontinued in some cases, we started an alternating Ubretid-i.v. Mannite treatment in 1968 (Gitsch & Tatra). 500 cc of a 10% Mannite (Leopold 3 Co., Graz, Austria) solution are administered by slow i.v. infusion for 1 or 2 hours after giving up to 1 000 cc tea orally and sodium chloride as well as potassium in the form of 500 cc Ringer solution. The resultant increase in diuresis is seen to correspond with an enhancement of the ureteral contraction rate, which is usually reduced postoperatively. On account of a possible interference with the electrolyte balance and a potential circulatory as well as renal overload, the use of Mannite as a substitute for Ubretid is, of course limited. Apart from that, Mannite infusions are indicated whenever the amount of urine voided daily drops under 800 cc in spite of normal fluid uptake.

Some of the prophylactic measures outlined also serve to prevent postoperative surgical complications. This ap-

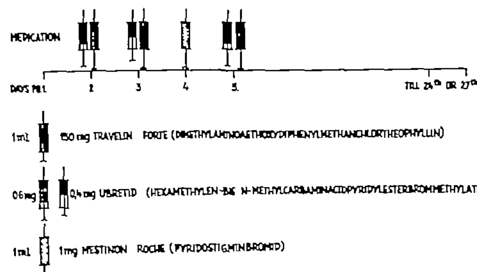


Fig. 2. Increase of ureteric and bowel motility and of bladder tonus by cholinesterase inhibition. Treatment schedule for a patient of 60 kg body weight.

Table I

A = control group
B and C = (groups of prophylaxis)

	A	%	B	C	% from B & C
Number of cases	140	—	232	61	—
Urinary retention than 3 weeks	27	19 28	8	3	3.75
Stricture of ureter	1	0.71	1	1	0 68
Pyelitis and pyelonephritis	13	9.28	7	1	2.73
Ureteric fistula					
Necrotic	7	5 00	1	—	0 34
After lesion			1	—	0.34
Death from urol. cause	1	0 71	—	—	—
Nephrectomy	2	1 42	—	—	—
Postoperative meteorism	10	7 14	—	—	—
Ileus	3	2.14	—	—	—
Mortality	5*	3 45	3	—	1 02

2 pulmonary embolisms, 1 cardio-vascular failure, 1 pyelonephritis, 1 paralytic ileus.
2 pulmonary embolisms, 1 peritonitis.

plies particularly to the prolonged cholinesterase inhibition both prevents both postoperative meteorism and intestinal paralysis, dreaded complication after prolonged surgical procedures.

(e) Elevation of the head end of the bed by 30 cm for 10 days to improve drainage f secretions and urine.

RESULTS

The following table illustrates the esults achieved with the measures described at the two Departments of Obstetrics & Gynaecology University of Vienna Medical School (figures from the Second Department do not extend to the period after I left).

140 radical Wertheim operations with selective lymph node removal performed at the Second Department of Obstetrics and Gynaecology between October 15 1954 and April 7 1959 served as a control group (A). This is compared with two prophylaxis groups. group B consisting of 23 patients on whom extended radical operations plus primary lymph node removal were performed at the Second Department between July 15 1960 and July 1 1968 (excluding the period from October 11 1961 until October 15 196_ when prophylaxis as described was not employed), and group C consisting of 61 cases treated by extended radical operations plus primary lymph node removal at the First Department since July 1 1968 The percentages are listed in Fig. 3

DISCUSSION

Although our method may seem somewhat over elaborate, the results obtained fully justify its application. Not only is morbidity substantially reduced and cure rates consequently improved, but also number of secondary operations necessitated by both surgical and urological complications is minimized. The need for this method is dramatically illustrated by 6 cases of Latzko operation performed at the First Department of Obstetrics and Gynaecology between May and July 1968. muscular activation as described was omitted and 2 patients developed uretero-vaginal fistulas, one of which spontaneously disappeared after 10 day course of Ubretid.

Among the last 45 patients of group B and the entire group C, who received Marcoumar (equivalent to Liquamar Organon in the USA) prophylactically (prothrombin time between 22 and 28 %) beginning with the third postoperative day there was not a single case of fatal pulmonary embolism. Similarly other thromboembolic complications were substantially reduced. Since 1970 proteinase inhibitor (Trasylol Farbenfabrik Bayer Leverkusen/Rhein, BRD) is used, as suggested by Mörl, for the first 5 postoperative days. Thus thromboembolism prophylaxis now begins during surgery and is continued postoperatively with Marcoumar (Trasylol and Marcoumar medications overlap beginning with the third postoperative day). The value of our method for reducing urological complications is indirectly corroborated by

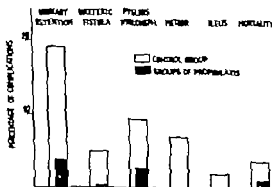


Fig. 3

Table II *Marcoumar prophylaxis* [3-(1 phenyl-propyl)-4-hydroxycumarine]

Abdom. radical operation	No. of cases	Thrombosis of the legs	Thrombosis of the pelvic veins	Pulmonary infarction	Pulmonary embolism deaths	Total number of thrombosis compl.
Group B with marcoumar	45					
Group C with marcoumar	61	2		1		
T tal marcoumar cases	106	(1.9%)		1 (0.9)		3 (2.8%)
Group B without marcoumar	187	7 (3 7%)	4 (2.1 %)	7 (3 7 %)	2 (1 1 %)	20 (10.6)

other authors. The results presented by Green (1966) also speak in favour of ureteric activity as an explanation of reduced complication rates: among 65 cases, the incidence of ureterovaginal fistulas was 1.5% Apart from the surgical positioning of the ureter this result is attributed to an improvement in peristalsis increased by the pulsations of the adjoining hypogastric artery Green's results were confirmed by Averette et al. (1969) and by Dunn.

On transposition of the ureters into the abdominal cavity after Novak, bowel activity which has an activating effect on the ureters also appears to be responsible for the reduced incidence of uretero-vaginal fistulas.

Complications caused by the method itself: Only two superficial haematomas were observed (caused by Marcoumar). Nausea or vomiting, due to the Ubretid medication were present in 5% of the patients, but only in the group with intramuscular injection.

The Vienna method offers the advantage that the drug-induced increase in ureteric activity does not result in a higher incidence of stenosis of the ureters and/or hydronephrosis compared to the surgical measures mentioned, particularly transposition of the ureters (Helbing)

In conclusion, we should like to emphasize that the success of our method depends on the meticulous application of every one of the measures described. If even one is insufficiently applied, prophylaxis may be inadequate and negative effects will soon manifest themselves.

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DETERMINATION OF 6-PGDH IN THE VAGINAL SECRETIONS OF PATIENTS WITH CARCINOMA OF THE CERVIX

A New Method of Collection of Vaginal Secretions

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Abstract. Determinations of 6-phosphogluconate-dehydrogenase concentration of vaginal secretions were made in 107 women, in 37 of whom carcinoma of the cervix was, subsequently histologically confirmed. The aim was to determine the value of this enzyme test in the detection of carcinoma of the cervix. In normal group there were 35% false positive findings, while in the carcinoma group there were 15% false negative findings. It is concluded that the method, at least in its present form, is not of value in the diagnosis of carcinoma. A new filter technique for collection of vaginal secretions was used, such as recommended for future similar studies.

Since the introduction of exfoliative cytology in the early diagnosis of carcinoma of the cervix, attempts have been made to supplement this with various other early detection methods. Changes in the pattern of vaginal secretions, especially in the quantity and nature of the enzymes, have been much studied.

This study is concerned with the evaluation of 6-phosphorgluconatedehydrogenase (6-PGDH) determinations by a new technique as a supplementary method in the diagnosis of early carcinoma of the cervix.

In 1960, Scarpelli & Pearse (1) demonstrated an increased pentose cycle activity especially at the periphery of squamous cell tumours, associated with the increased nucleic acid synthesis of the tumour cells.

Bonham & Gibbs, 1960 (2), published promising results from 6-PGDH determinations in patients with genital carcinoma. In 4 women with carcinoma of the cervix, 12 with carcinoma of the body of the uterus, and 8 with malignant tumours of the vulva or vagina, values were elevated when compared with a control study of 94 normal women.

However Nerdrum, 1964 (3), studied 150 patients, and found elevated 6-PGDH secretion in a significant number without demonstrable genital carcinoma. He also found normal values in patients with histologically confirmed carcinoma of the cervix. Furthermore, he demonstrated increased production of the enzyme in women with trichomonas vaginitis.

Glucose-6-phosphatedehydrogenase activity in vaginal secretions was studied by Lyng, 1964 (4). Activity was increased in presence of cervical carcinoma, but was not specific. Increased activity was also observed in trichomonas vaginitis and during resolution of vaginal lesions, e.g. erosions.

Cameron et al., 1965 (5) made 6-PGDH determinations in 2 480 women. In all patients with established carcinoma of the cervix, enzyme secretion was increased, but was normal in 50% of patients with carcinoma in situ. In normal women, enzyme secretion was also found to be dependent on age. Thus in 30% aged around 30 years, and 70% aged around 60 years, elevated values were found.

Goldberg et al., 1968 (6), made preliminary examination of 55 women, 25 of whom had cervical carcinoma. Twenty-three of the latter had elevated 6-PGDH secretion, but only 5 of the 30 normal controls.

Table I *Patients with carcinoma of the cervix*

Stages	1a	1b	2	2b	3	4
No of pats.	5	8	6	6	9	3

Table II *Age distribution of patients*

Age of pats.	20–30	30–40	40–50	50–60	60–70	70–80
Without carcinoma of the cervix	41	19	4	3	1	2
With carcinoma of the cervix	0	8	12	12	1	4

METHOD

Previous investigators have aspirated vaginal secretions through a pipette introduced into the vagina.

The device used in this study was an ordinary cigarette filter approximately 0.75 cm long. When such a filter is introduced into the vagina, it absorbs secretions by capillary attraction.

The filter is first impregnated with a solution of 25% human albumin. It is attached to a 15 cm nylon rod to facilitate introduction into the vagina. For storage purposes, it can be completely capped and dry frozen to preserve the albumin. If such devices were issued to women, samples of vaginal secretions could be taken at home, and preserved in the interim period, before return t the laboratory simply by replacing the cap aft r use.

Laboratory processing is as follows: with a nail, the filter is pushed free from the nylon rod int a small plastic tube. Secretions are extracted with 1 ml glycylglycin buffer (pH 9), and 1 ml 0.1 M magnesium chloride. Three holes are bored in the capped plastic tube which is then fixed in larger tube and centrifuged. *The enzyme containing fluid is centrifuged out through* the holes, into the larger tube and after 10 min, sufficient is available for activity determination. Enzyme co centration is then calculated as by Bonham et al. (2). Concentration is expressed as μM substrate reduced/min/g vaginal secretion.

Clinical series

The study comprises samples from 107 women. Vaginal secretions were collected at the first gynaecological examination, before definitive diagnosis had been made and *6-PGDH concentrations were determined.*

Subsequently after biopsy and dilatation and curettage, diagnosis of carcinoma of the cervi was histologically confirmed in 37 women. Diagnoses in the remaining 70 women were amenorrhoea, genital prolapse pregnancy sterility metrorrhagia, and other similar conditions.

Table I shows the staging of the carcinoma in the 37 patients, and Table II gives the patients ge distribution.

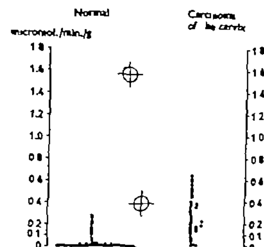


Fig 1 Concentration, determined at 25°C, of 6-PGDH in vaginal secretions.

RESULTS

The findings are recorded in Fig. 1

All values under 0 1 μM/mm/g were arbitrarily taken as normal. By this standard 24 of the 70 patients without malignant disease had elevated 6-PGDH values. That is to say that incidence of false positives was 35%

Of the 37 carcinoma patients, values were clearly elevated in 31 but normal in 6 That is to say that the incidence of false negatives was 15%

*If the arbitrary normal standard were set lower than 0 1 μM/min/g, the incidence of false posi*tives would be very high.

DISCUSSION

The results of this study confirm those of earlier studies that there is often an increase in pentose cycle activity with carcinoma of the cervix

The advantage of a reliable chemical test demonstrating changes in vaginal secretions which *reflect tumour presence and activity is clear* Analysis of the secretions could be made standard laboratory practice on automatic machines. Mass screening would therefore be possible By comparison, screening on this scale by exfoliative cytology studies is impossible because of the lack of experienced personnel.

This study demonstrated generally increasing 6-PGDH concentrations in the vaginal secretions of patients with carcinoma of the cervix, but there was a false negative incidence of 15%

There were also false positive findings in 35% of normal women.

No account was taken of other conditions which might give elevated enzyme production—e.g. trichomonas vaginitis and cervical erosions (4). The intention was solely to study the reliability of the test in detection of carcinoma, irrespective of other genital pathology.

It cannot be concluded that the test in its present form is of value, partly because it is not sufficiently specific, but largely because of the high incidence of false negative findings.

The method used for collection of vaginal secretions and testing, seems particularly to recommend itself in any future studies.

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Menorrhagia may be caused by an increase in local fibrinolytic activity Cyklokapron reduces menorrhagic haemorrhages by an average of 50 %

Women with average menstrual blood losses of over 80 ml have higher concentrations of plasminogen activators in the endometrium than those with lower blood losses The resultant increase in local fibrinolytic activity is inhibited by Cyklokapron The recommended dosage of Cyklokapron in menorrhagia is 1 g 3–6 times daily for 3–6 days With a dosage of 3 g daily Nilsson and Rybo noted reductions in bleeding of 38 % compared with control cycles. With twice this dosage bleeding was reduced by 51 %. None of the 36 patients participating in the trial were obliged to discontinue treatment as a result of side-effects.

Reference. NILSSON L., RYBO G.. Treatment of menorrhagia with an antifibrinolytic agent, tranexamic acid (AMCA) A double blind investigation Acta Obstet Gynecol Scand. 46 (1967) p 572

the fibrinolytic system

ACTIVATORS
tissue activators
lab blood activators
streptokinase
urokinase
trypsin

plasminogen
↓
plasmin
↓

INHIBITORS
inhibitors of plasminogen activators
(= urokinase inhibitors)
EACA, AMCA
α2 – macroglobulin
α1 – antiplasmin
Split products

fibrinogen
fibrin
factor V
AHF
other proteins

HMWS
↓ ↓
D E



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RENAL ANGIOGRAPHY IN THE FOLLOW-UP EXAMINATION OF TOXAEMIA OF LATE PREGNANCY

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Abstract. A series of 35 patients with toxaemia of pregnancy is presented. These patients were subjected to renal angiography in order to diagnose any renal artery changes. The patients formed part of a series of 139 toxaemic patients, all of whom had been followed up by intravenous pyelography with normal renal findings. One patient showed unilateral obstruction of the main branch of the renal artery obviously of importance. Another patient was found to have a saccular aneurysm in the abdominal aorta. These changes were recorded in the group of patients with history of hypertension preceding pregnancy and with superimposed toxaemia of late pregnancy. The groups with pure preeclampsia showed no pathological findings, and renal angiography in these groups shed no additional light on the aetiology of toxaemia.

Pre-eclampsia may be secondary to renal disease or preexisting hypertension. Hocinsh & Stöckli (1959) carried out intravenous pyelography and renal biopsy as part of the follow-up examination of a series of women, whose pregnancies had been complicated by severe preeclampsia, and found abnormalities in 69%. The number of abnormal findings in the toxaemic series followed up by Koskela & Pystynen (1968) by means of intravenous pyelography only was almost 50%. The number of the hypertensive conditions of pregnancy in which the aetiology can be ascertained depends essentially on the methods applied at follow-up examinations.

Renal angiography during the puerperium on patients, whose pregnancy had been complicated by a hypertensive condition (Landesman et al., 1961) seemed to offer a possibility of reducing the number of cases in which the aetiology remained uncertain. Angiography revealed an abnormality of the renal artery in two out of their ten toxaemic patients. According to these authors, such abnormalities of the renal artery may contribute towards the development of certain toxaemias. Aortography has also been used in examining patients with toxaemia of unknown aetiology post-partum (Clemetson, 1960; Gordon & McKay 1964). Clemetson found aortic hypoplasia in more than half the group of patients with severe pre-eclampsia.

So far as we have been able to trace in the literature, the paper by Landesman et al. (1961) is the only one endeavouring to ascertain the role of an abnormality of the renal artery in the aetiology of toxaemia of pregnancy. We therefore considered it useful to apply renal angiography in the follow-up examination of a number of patients, whose pregnancy had been complicated by a hypertensive state.

The Series

The incidence of hypertensive conditions in pregnancy among the mothers of the Department of Obstetrics and Gynaecology of Oulu University is 12–13%. Toxaemic mothers treated in 1965–1967 were invited to come for re-examination after the puerperium. The attending patients had several investigations, including intravenous pyelography 2–6 months after delivery. Of the series of 139 toxaemic patients whose intravenous pyelogram revealed no renal anomalies and no inflammatory or ureteric changes, 35 agreed to undergo renal angiography (Table I). These 35 patients constitute the present series. The clinical classification of their toxaemia during pregnancy can be seen from Table I.

Table I. *Distribution of the hypertensive conditions during pregnancy by clinical symptoms, and the series followed up with renal angiography*

Clinical diagnosis	Number of patients followed up by iv pyelography with normal findings	Follow up by renal angiography	Pathological findings in renal angiography
I Mild pre-eclampsia	43	3	—
II. Severe pre-eclampsia	57	10	—
III. Eclampsia	10	5	—
IV Hypertension complicated by toxaemia of late pregnancy	5	16	2
V Hypertension not complicated by toxaemia of late pregnancy	4	1	—
Total	139	35	2

The hypertensive states of pregnancy were divided into groups according to the recommendation issued by the American Committee on Maternal Welfare as modified by Werkö in 1948 The patients with severe pre-eclampsia had continuous proteinuria, their blood pressure exceeded 160/110 mmHg, they showed some oedema, and they had subjective complaints. The same was true for the patients with mild pre-eclampsia except that their blood pressure was below 160/110 mmHg, and they had no subjective symptoms.

METHOD

Renal angiography was carried out by transfemoral aortic catheterisation using Seldinger's (1953) technique Under local anaesthesia with 0.5% lidocaine and after premedication with scopomorphine and decadron, a 50 cm long teflon catheter with a tip modelled to equal a metal catheter no. III was introduced to the level of the origin of the renal arteries. The end of the catheter was blocked with metal stopper (Virtama, 1963). 40 ml of 60% Urografin® (Schering) contrast medium was injected with Gidlund pressure syringe using a pressure 1 5 kp/cm² A six-valve machine and Elema-Schönander film exchanger (35×35 cm) were used. The film focus distance was 100 cm. A 3 -exposure film (Kodak Blue Brand), duration about 9 sec, was used.

RESULTS

The results can be seen from Table I. The renal angiograms showed no pathological findings in the pure pre-eclampsia groups. Pathological findings were recorded in two of the 16 patients whose pregnancy was complicated by hypertension with superimposed toxaemia of late pregnancy One of the 2 patients had an obstruction of the main branch of the left renal artery (Fig. 1) She was a Para VII, aged 41 whose blood pressure had been elevated since the pregnancy began She also had a history of toxaemia in a previous pregnancy The symptoms of pre-eclampsia started in the 37th week of gestation. The delivery was by Caesarean section 1 week after admission because of intrauterine foetal asphyxia. The child survived. At the follow-up examinations the patient's blood pressure was 170/120 and 185/120 she had no proteinuria, creatinine was 1 0 mg/100 ml and the urinary sediment was normal. The intravenous pyelogram was normal too but renal angiography revealed a stenosed left renal artery (Fig 1).

In the other patient, the finding was a saccular aneurysm of the abdominal aorta, obviously of no significance in the aetiology of toxaemia.



Fig 1 Renal angiogram of patient with left renal artery stenosis.

DISCUSSION

The examination revealed no abnormal findings in either the mild or the severe pre-eclampsia groups. Despite the small number of patients followed-up by renal angiography it may be concluded that the proportion of renal artery anomalies associated with pre-eclampsia cannot be great. Half of the total number of patients in the eclampsia group attended the follow-up examination, and no pathological changes in the renal arteries were recorded among them. According to the present results, renal artery anomalies play no part in the aetiology of the toxaemia itself.

The two pathological findings were discovered among the group of hypertensive patients whose late pregnancy had been complicated by symptoms of superimposed pre-eclampsia. Of the patients in this group more than half attended the follow-up examination (16 out of 25). The only obstruction of the renal artery was found in group identical to that in which Landesman et al. (1961) recorded their two pathological cases.

All the patients of this group showed persistently raised systolic and diastolic blood pressures at the follow-up examinations. In our opinion, the present series shows that renal angiography as part of the follow-up examination of hypertensive pregnant patients need only be recommended if the blood pressure remains at a high level after delivery

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THE SIGNIFICANCE OF THE FOETAL ELECTROCARDIOGRAM DURING LABOUR WITH DETAILED REPORT OF ONE CASE

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Abstract. The preceding study reported continuous monitoring of the foetal heart rate (FHR) during labour in 86 cases. In 60 cases assessment of the FHR was based upon the foetal electrocardiogram (FECG) recorded by means of a lead from the presenting foetal part after rupture of the membranes. In 31 instances the FECG was of such good quality that the entire ECG complex was recorded distinctly. Of these deliveries 16 were entirely normal, while in 7 the infant was born with the cord around its neck and 8 were complicated by placental dysfunction. The average values for the normal FECG are presented. It is noted that the T wave is negative only in exceptional cases. In one case in which the cord was around the neck there was a period of extrasystoles and in another case transient notching of the initial complex. In placental dysfunction the T waves are usually negative without the infants being at all depressed. One case of severe placental dysfunction is described in detail. The foetus died during labour, and the FHR showed pronounced changes. In the electrocardiogram the T waves grew deep and peaked, and terminally there was a prolonged conduction time and notching of the initial complex. It is concluded that the FECG is a poor indicator of foetal welfare during labour.

While it is generally accepted that the foetal heart rate (FHR) is a good indicator of foetal well being during pregnancy and labour, the diagnostic value of the foetal electrocardiogram (FECG) is controversial.

Since Cremer (1), in 1906, first demonstrated the FECG as a very small wave in the lead from the maternal abdomen, great efforts have been expended on improving the quality of the recorded signal.

Technical difficulties have been immense. The signal obtained from the abdomen was very faint and unstable, often submerged by extraneous noise. As a rule, only the initial complex was visualized, always far fainter than the inevitable maternal ECG.

By modifying electrodes and filtration the quality has been considerably improved in the course of the past 10 years, but still it is not possible to obtain all details of the electrocardiogram by an abdominal lead except in cases where a noiseless FECG is obtained by computer analysis of many ECGs. However, this requires an extremely complicated and expensive apparatus and has been performed only in few hospitals, so this method is unlikely to become of clinical importance.

During labour the abdominal recording is greatly impeded by uterine contractions and maternal restlessness. In this respect the *vaginal electrode* designed by Hon (3, 6) is a great advance. This electrode is applied to the presenting foetal part after the membranes have ruptured. The FECG thus obtained includes all electrocardiographic details and is moreover stable, at least between contractions. As a rule, the maternal ECG can barely be discerned on the curve.

While with the abdominal lead the FECG may alter during labour because of a changed position of the foetus, the FECG obtained with the vaginal lead is independent of the foetal position. The abdominal FECG is most reminiscent of the Standard Lead II (8), while the vaginal FECG is only partially like the records obtained with Standard leads (5).

There has been a great deal of discussion as to whether the vaginal or abdominal recording of the FECG is of any clinical value. Opinions have been extremely divided, especially between Larks and Hon. While Larks (7) claims that the FECG complex may disclose intrauterine difficulties which do not give rise to changes in the FHR, Hon (4) believes that any changes of the

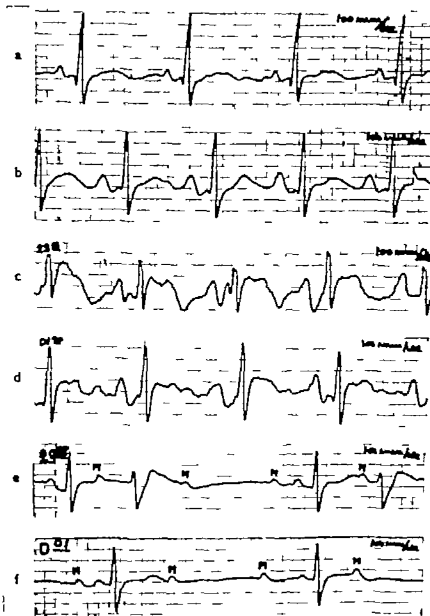


Fig 1 Examples of FECG recorded during labour by direct vaginal lead. (*a, b*) Entirely normal FECGs. (*c d*) Cord around the neck. During one period ( ) notched R, elevated ST and deep T later (*d*) a normal FECG. (*f*) Cord around the neck. A short period ( ) of ventricular extrasystoles, but thereafter (*f*) a normal FECG

FECG complex are preceded by marked changes in the FHR.

Most interest attaches to the question whether the FECG complex exhibits characteristic changes in the event of foetal hypoxia. In this condition Southern (11) found high P waves, a prolonged PQ a depressed ST segment, and in some cases negative T waves, while Hon (4) found high P waves and a shortened PQ to be the most constant findings. In rabbit foetuses subjected to hypoxia Gelli & Gyulai (2) found first brady cardia, followed gradually by a prolonged PQ depression of the ST segment, and negative T waves. Romney et al. (10) concluded that changes in the FECG constituted a poor indicator of foetal hypoxia. None of the above-mentioned authors found changes in the QRS complex during hypoxia. In dying foetuses Hon (4) has found the QRS complex to be unaffected even by very severe hypoxia changes of the QRS complex were not demonstrated until immediately before *foetal death*

However changes in the QRS complex may occur during labour without any foetal distress (9). Sustained changes may indicate congenital heart disease, while sporadic QRS changes may occur as a result of compression of the cord.

PRESENT INVESTIGATION

Continuous monitoring of the FHR has been carried out during labour in 86 cases, cf. the preceding study In 60 cases the monitoring was based upon FECG lead

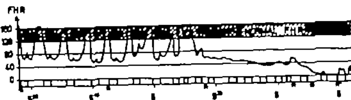


Fig. 2. (1323/68). The dying foetus. Reconstruction of the FHR curve during the last 50 min before death. Deep early and deep late decelerations, and during the last 25 min severe bradycardia. The times of the FECGs in Fig. 4 are marked by asterisks.

from the presenting foetal part by means of the vaginal electrode designed by Hon. For details of the apparatus and procedure the reader is referred to the preceding study

The electrocardiogram was primarily intended as means of demonstrating the heart rate, but in 49 cases it was recorded on paper. In 31 of these cases the quality was so good that the entire FECG complex was clearly apparent. Of these deliveries 16 were entirely normal, while in 7 the cord was around the neck of the foetus and 8 were complicated by placental dysfunction. Particular attention will be given to cases in which the foetus died during labour and in which distinct electrocardiographic changes were found

NORMAL LABOUR

This includes also the 7 cases in which the cord was around the neck, i.e. a total of 23. Figs. 1 and *b* exemplify entirely normal electrocardiograms. It will be seen that the quality is excellent, all the details of the complex being clearly outlined.

The following average values were found for the individual components of the complex.

P/RS. 1 7 (1 2–1 14)
PQ: 0.11 sec (0 09–0.14)
QRS 0.07 sec (0.05–0.10)
ST elevated in 4/23 isoelectric in 19/23
T positive in 4/23 isoelectric in 17/23
negative in 2/23

Absolute measures of the height of the individual waves cannot be stated, as the amplification varied from case to case. Therefore, the ratio between the height of the P wave and the R+S waves is given. There is a marked dispersion, i.e. the height of the P waves varies widely and may be up to half the height of the QRS complex.

PQ and QRS are fairly constant from case to case. It may be difficult to decide which is the ST segment and which the T wave. It is worth noting that inversion of the T wave was seen only in exceptional cases.

While there was some variation in the appearance of the FECG complex from foetus to foetus, its appearance remained largely unchanged throughout labour in all except two cases. In one of these cases (Figs. 1 and *d*) there was a period (*c*) of notched R waves, greatly elevated ST and deep, peaked T waves. Later (*d*) the FECG was normal. The infant was unaffected at birth, but had the cord around its neck.

In the other case (Figs. 1 *e* and *f*) there was a short period (*e*) of coupled ventricular extrasystoles. The infant was delivered 20 min later with the cord around the neck and slightly depressed. This delivery has been reported in the preceding paper (case rec. 1656/58).

PLACENTAL DYSFUNCTION

Among this group of 8 patients one foetus died during labour The severe FECG-changes found

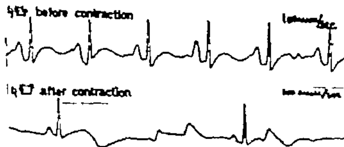


Fig. 3 (1323/68) FECG before and after contraction at hours 4.56 and 4:57 Before the contraction the FECG is normal, while after the contraction the T waves are negative. The heart rate is fast before the contraction (tachycardia) and very slow after (late deceleration)



Fig 4 (13.3/68). Changes in the FECG during the last 50 min before foetal death. Gradual development of a deep and peaked, negative T wave After the onset of severe bradycardia (cf. Fig.) prolongation of the conduction time and notching as well as widening of the QRS complex.

in this case will be described in the following sections. Another infant was severely depressed at birth and later died of anoxic damage. In this case the electrocardiogram did not exhibit any abnormality

In the remaining 6 cases the infants were entirely unaffected at birth. On comparison of the FECGs in these cases with the FECG known from normal labour no changes in P PQ and QRS were detected. On the other hand, ST was raised in a larger number of cases (5/6), and—the main difference—the T waves were negative in practically all cases (5/6) and deep and peaked in half the foetuses.

ELECTROCARDIOGRAM OF THE DYING FOETUS

(Case rec. 1323/68.) The course of the pregnancy and labour has been described in detail in the preceding paper The FHR curve showed, throughout labour changes indicating severe hypoxia. Fig. 2 is a reconstruction of the FHR curve during the last hour before death. During the last 25 min there was very severe bradycardia (direct action of hypoxia upon the myocardium), and during the last minutes few heart beats.

Fig. 3 shows the electrocardiogram traced before and after a contraction, immediately before that part of labour which is illustrated in Fig. 2. The FECG prior to the contraction is normal, while after the contraction the T wave has become negative. The PQ is shorter than normal both before and after the contraction (0.08–0 07 sec as compared with 0 11 sec normally). This may have been caused by a small heart (birth weight 1 1150 g), the conduction time being proportional to cardiac size.

Fig. 4 exhibits the changes in the electrocardiogram during the last 50 min before foetal death. The times are marked by asterisks on the FHR curve in Fig. 2.

It is apparent that the PQ remains unchanged until the onset of severe bradycardia. Thereafter PQ becomes prolonged beyond the normal range (to 0 16 sec). The QRS complex, too, is unchanged until after bradycardia sets in. Thereafter it widens and gets notched. The ST is raised throughout, and the T wave gradually grows deeper and more peaked

DISCUSSION AND CONCLUSION

Foetal electrocardiography is considered applicable for demonstrating a live foetus (or foetuses) congenital heart disease and for recording the foetal heart rate. On the other hand, it is of limited value as a parameter of foetal welfare during pregnancy and labour This view accords with our experience. True our material is small, but the quality of the electrocardiograms obtained with the vaginal lead is far better than that of FECGs in the abdominal lead on which most previous reports have been based. Owing to this difference in the lead it is difficult to make further comparisons.

On the basis of our case of severe hypoxia, we feel that the QRS complex is largely unaffected by hypoxia, not showing any changes until irreparable changes in the myocardium have occurred.

The size of the P wave normally varies so much that in our opinion its height is of no diagnostic value. The PQ is normally unchanged during

labour and in the event of hypoxia it does not become prolonged until terminally

ST in our lead, was either raised or iso-electric, never inverted. ST does not appear to alter during hypoxia. On the other hand, hypoxia causes distinct change of the T wave, which gradually grows deeper and more peaked. Under normal conditions the T wave is only exceptionally negative. It is striking that in cases of placental dysfunction the T waves are usually negative without the infant being depressed at birth. We are unable to advance any explanation of this phenomenon. A negative T wave presumably indicates some cardiac strain which need not be life-threatening.

It may be concluded that severe hypoxia causes distinct changes in the FECG particularly in the conduction time and in the T wave, and that changes in the QRS complex do not occur until terminally Similar changes in the T wave may occur without any threat to the foetal health. Compared with the FHR, the FECG is a poor indicator of foetal welfare during labour

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CONTINUOUS MONITORING OF THE FOETAL HEART RATE AND UTERINE CONTRACTIONS DURING LABOUR

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Abstract Foetal welfare during labour may be monitored by continuous recording of the foetal heart rate (FHR) and uterine activity The nomenclature relating to the FHR curve is outlined and the apparatus described. The FHR monitoring was based upon the foetal phonocardiogram (abdominal) or electrocardiogram (vaginal). 86 patients were monitored during labour 34 of them because of an abnormal pregnancy or meconium-stained amniotic fluid. In normal delivery (33 patients) early decelerations were observed during the second stage of labour in one-half of the cases and bradycardia during the last minutes before delivery in one-quarter When the cord was around the neck (15 patients) variable decelerations occurred in one-quarter and frequently deep early decelerations during the second stage of labour In two of these cases the foetus developed hypoxia. Meconium-stained amniotic fluid (10 patients), especially when combined with placental dysfunction, is a poor prognostic sign. One infant showing this sign died during delivery The FHR curve showed tachycardia, minimal irregularity and deep late decelerations. Another infant had severe brain damage and died later. Its FHR curve had shown increasing basal FHR and irregularity on the verge of the minimal. Lastly in a case of deep late decelerations the infant was unaffected at birth. Within the total series FHR abnormalities were seen in 14 cases; 9 of the infants were unaffected, while 5 were depressed or dead. The FHR curve was normal in 72 cases; 71 of the infants were unaffected and only one depressed at birth. It is concluded that late decelerations may be a sign of hypoxia, especially when deep and combined with tachycardia. The most serious sign that the life of the foetus is in danger is cessation of the irregularity on the FHR curve.

Until a few years ago the only possibilities of evaluating foetal well-being during labour were auscultatory counting of the foetal heart rate (FHR) between contractions and assessment of the colour of the amniotic fluid after rupture of the membranes. Both are of limited value. It was demonstrated by Benson et al. (2), on the basis of approximately 25 000 deliveries, that in fact there is no single auscultatory indicator of foetal distress, and Hon (10) ascertained that obstetricians are unable to count the FHR accurately More over, Wood & Pinkerton (25) found that admixture of meconium in the amniotic fluid does not increase perinatal mortality

During the past 15 years methods for continuous recording of the FHR have been developed. Besides determination of the acid-base status of the foetal blood, simultaneous recording of the FHR and the uterine contractions is to-day the best means of monitoring foetal well-being during labour

PRINCIPLES OF RECORDING FHR AND CONTRACTIONS

Continuous monitoring of the heart rate may be based upon a recording of the foetal electrocardiogram or phonocardiogram. In the former case the R wave of the electrocardiogram is used as a trigger for counting the heart rate, in the latter case the excursions on the phonocardiogram corresponding to the two heart sounds. In both instances it is the instantaneous heart rate which is recorded.

Recording from the foetal electrocardiogram is based mainly upon the numerous studies of Hon (10, 11 12, 13 14 15 16, 17). Hon designed an electrode which affords, when applied to the presenting foetal part after rupture of the membranes, a pure foetal electrocardiogram which is strong (approx. 200 μV) and stable, also during contractions. On the other hand, it has not yet been possible to use the foetal electrocardio-

Table I *Difference between the nomenclature used by Hon and Hammacher concerning irregularity and periodic deceleration*

	Irregularity (oscillation)				Periodic deceleration		
Hon	Minimal irregularity	Moderate irregularity		Marked irregularity	Early deceleration	Late deceleration	Variable deceleration
Hammacher	Osc. type 0 (0–5)	Osc. type 1 (5–10)	Osc. type 2 (10–25)	Osc. type 3 (more than 25)	Dip I	Dip II	Dip 0 Comb. Dip I+II
	Silent	Narrow undulatory	Undulatory	Saltatory			

gram with the lead from the maternal abdomen, as it is far weaker (approx. 20 μV), not stable, and invariably far fainter than the inevitable maternal electrocardiogram

The use of the phonocardiogram as a trigger for the FHR monitoring is reasonable, as this signal is strong (approx. 10 000 μV) However the problem has been to filter out extraneous noise This problem has been solved by Hammacher (7) who used a selection principle, and thereby this method has been rendered applicable clinically

The necessary simultaneous recording of the contractions may be done most simply by marking on the FHR curve the contractions as stated by the patient. A better method is external measurement of the contractions by a capsule fastened by a belt on the abdomen. The most accurate recording of the contractions is obtained by inserting a catheter into the uterus—as a rule by the vaginal route after the membranes have ruptured. This method was worked out on the basis of Caldeyro-Barcia's studies (1) The exact measurement of the intrauterine pressure obtained thereby has proved of importance in interpreting the various changes in the FHR.

FHR—NOMENCLATURE AND CLINICAL SIGNIFICANCE

It has proved difficult to arrive at a uniform interpretation of the various changes in the FHR curve, and the nomenclature has been altered several times. The nomenclature in the present paper is that used most recently by Hon (16) In all essentials it is in agreement with Caldeyro-Barcia's, while in some respects it differs from Hammacher's (8) (cf. Table I)

The heart rate curve is characterized by (1) the basal FHR, (2) the degree of irregularity of the FHR, and (3) periodic or sporadic accelerations or decelerations.

Basal FHR

The basal heart rate is the average rate between contractions, without any accelerations or decelerations. Normally it is between 120 and 160 beats/min. A basal heart rate below 120 and above 160 is classified as bradycardia and tachycardia respectively which again may be moderate or marked (< 100, > 180)

Bradycardia. Bradycardia is usually caused by increased vagal tonus. True bradycardia is rare and of no major clinical importance (20). Bradycardia may be a sign of heart disease in the foetus.

Tachycardia Many studies (4 5 19 21 26) have demonstrated an inverse proportionality between the pH in the foetal blood and the basal FHR, i.e. a decreasing pH and tachycardia going together When hypoxia develops an acidotic condition (pH < 7.20) arises, and the basal FHR rises because of an increased sympathetic tonus and perhaps release of adrenaline-noradrenaline.

However tachycardia may be observed also in entirely normal foetuses for varying periods during labour but a slowly increasing tachycardia is almost invariably a sign of foetal hypoxia.

Irregularity of the FHR

Normally there are slight variations between the individual heart beats, causing an irregularity of the FHR curve which thus acquires a certain width depending upon the degree of the irregularity For the various nomenclatures, cf Table I.

Normally the degree of irregularity has an

itude of 5–25 beats/min. Marked irregularity litude exceeding 25 beats/min) indicates ly compromised circulation in the cord and little significance. On the other hand, mini- irregularity (amplitude 0–5 beats/min) has ed of great significance. It indicates failure ne cardiovascular fine regulation and occurs in re intrauterine hypoxia. Hammacher (8), in icular has called attention to the minimal ir larity which he calls oscillation type 0.

The irregularity was not recognized until pos- ility of recording the *instantaneous heart rate* d been opened up. By auscultation it is not sible to hear the variation between the in- vidual heart beats, and counting over a lengthy riod will mask the phenomenon.

ccelerations and decelerations

his means transient increase or decrease from ne basal heart rate. While the irregularity repre- nts changes between the individual heart beats, re are now dealing with changes in the FHR urve of / to several minutes duration. Ac celerations and decelerations are generally related o the contractions (periodic), but may also be observed without such relation (sporadic).

Sporadic accelerations occur after foetal move- ments. Like sporadic decelerations, they are not *attributed with any clinical importance. Accelera* tions during contractions may indicate incipient hypoxia, but are frequently observed periodically *during entirely normal labour* and are not be- lieved to be f much value. On the other hand, periodic decelerations have proved essential in evaluating *foetal well-being during labour*. There are 3 different types, according to their time rela- tion to the contractions (cf. Fig. 1):

(*a*) *Early deceleration*. This occurs a few sec- onds after the onset of a contraction of which t is a reflection. The basal FHR is within the range of normal. Early deceleration is caused by in- creased intracranial pressur which increases the vagal tonus by direct action upon the vagus centre Early decelerations are very common dur ing labour especially during the second stage where contractions are strong and the foetal head is compressed in the true pelvis. Their presence is of no clinical significance.

(*b*) *Lat deceleration*. In these cases the de- crease in FHR does not start until the contraction has lasted for some time, and the rate has not

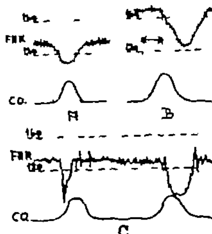


Fig 1 The three types of periodic deceleration. (A) Early deceleration, reflection of the contraction. (B) Late deceleration The fall in FHR is delayed in relation to the increase of intrauterine pressure. Basal FHR above the range of normal. (C) Variable deceleration, in appearance as well as in relation to the contraction. The fall is abrupt and marked.

returned to its initial level until more than half a minute after the contraction. Late deceleration, too is reflection of the contraction, but de layed in relation to it. *The decrease need not to be less than 120, but may be down to 60* beats/min. The basal FHR is usually above nor mal or at the upper end of normal. In many previous papers on bradycardia and hypoxia (3 9 11) there has not, according to the present nomenclature, been a question of bradycardia, but of late deceleration. Late deceleration is sign f utero-placental insufficiency The mild degrees constitute the earliest sign of hypoxia and would not be detected by auscultation, as the heart rate exceeds 10 beats/5 sec all the time.

(*c*) *Variable deceleration*. In these cases the deceleration varies in appearance as well as in onset from contraction to contraction. The de crease is often abrupt and usually to less than 100/min, and it may be sustained also after the contraction. *Variable deceleration is caused by* compression of the cord.

APPARATUS

Since May 1968 the Cardiotocograph designed by Hammacher in collaboration with the firm Hewlett-Packard has been in use in our department. This instrument is combined FHR and contraction meter based upon phonocardiography and external tocography (cf. Fig. 2).

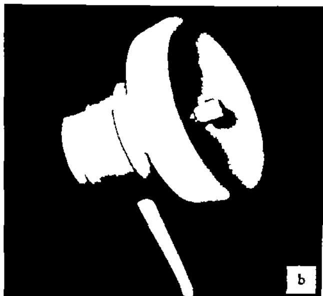


Fig 2 ( ) The Cardiotocograph with the two-channel writer (*b*) The combined transducer w th the pressure sensiti e capsule in the middle.

In the Cardiotocograph a microphone and pressure sensiti transducer are combined n capsule which is secured by a belt on the maternal abdomen where the heart sound is most distinct. Th. foetal heart sound is audible in loudspeaker and is also converted to the instantaneous heart rate which is isible on scale and recorded, *together with the contractions*, on a two-channel writer The recording is done on heat-sensitiv paper which moves at speed of 1 or cm min.

Apart from this, direct electrocardiographic recording of the FHR was employed. T this end we used the vaginal lectrode designed by Hon (14, 15) in collaboration with the firm Corometrics (cf. Fig. 3). This elec trode is applied to the presenting part of the foetus af ter the membranes ha e been ruptured. It is bipolar lead, the negative electrode on the foetus and the positiv one, at a distance f about 1 cm, in contact with the mother by w y of the secretions in the agina. With silver–silver chloride electrodes and perfect isolation, this method gives *pure foetal electrocardiogram* which is applicable for recording the FHR under all conditions. The electrode, somewhat like an agraffe, is applied with special forceps, either directly —better—through the amnioscopy tube used for taking micro blood specimens. Its application requires, part from ruptured membranes, cervical dilatation of 3 cm

The signal is amplified pre-amplifier and an electrocardiograph where the ECG may be recorded and *from which the amplified signal is conveyed to the car* diotocograph where it may be employed instead of the phonocardiographic signal for recording the FHR.

The necessary simultaneous recording of uterine activity was done either according to the patient statements by marking the onset and termination of the contraction on the FHR curve or by using the combined transducer f the Cardiotocograph for pure recording f the contractions.

PRESENT INVESTIGATION

During the period from May 1968 to December 1969 continuous monitoring of the FHR and contractions was performed in a total of 86 cases. In 26 the FHR was recorded by phonocardiography, in 34 by electrocardiography and in 26 by a combination of both. The average period of monitoring was 2 hours, while the longest continuous monitoring lasted more than 10 hours.

In 28 instances microblood analyses were done on foetal blood once or twice during labour. These analyses were done by the method of Saling (24). In this connection, the determination of the pH is of particular interest. It was normal (above 7.20) in all cases but one.

The condition of the newborn was assessed by Saling's scoring system, allotting from 0–12 points (0–3 for tension in the cord, 0–3 for skin colour, 0–3 for tonus, and 0–3 for respiration).

The reasons why the monitoring was carried out were as follows: meconium-stained amniotic fluid in 10 cases, combined with placental dysfunction in 4. Another 9 patients had placental dysfunction. 5 patients were past term, 9 were Rhesus immunized, and 2 had chronic renal disease. In the remaining 52 cases no abnormalities were found at the time when the monitoring was started, and the motivation was merely to collect normal material.

Particular attention will be devoted to three groups of patients: first, 33 patients with an entirely normal pregnancy and delivery. Secondly, 15 patients with normal pregnancy and delivery but the infant had the cord around its neck. Thirdly, 10 patients with meconium-stained amniotic fluid.

Concerning the other patients, it may be mentioned that the 9 patients with placental dysfunction, the 5 who were past term, and the 2 with chronic renal disease did not exhibit abnormal FHR changes during labour and the babies were unaffected. In a patient with a greatly depressed Rhesus infant there were repeated, but slight, late decelerations, and the infant received 6 points in the Saling score. In the other Rhesus deliveries there were no special abnormalities. In one case of the normal series deep late decelerations and an increasing basal FHR occurred in the first stage of labour. On this indication, a Caesarean section was carried out. The infant got 7 Saling points. Another 2 cases of the normal series had Caesarean sections (cephalopelvic disproportion). Lastly one infant was born with severe malformations from which it later died. The FHR curve had shown normal appearances except for tachycardia, and the infant was unaffected at birth.

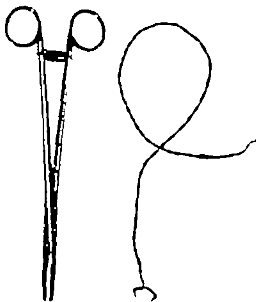


Fig. 3 Hon's vaginal electrode. The negative electrode, which looks like an agraffe, is applied by the special forceps to the presenting part of the foetus. The positive electrode, which is a small spiral wire, is placed about 1 cm from the agraffe.

Normal pregnancy and delivery

All the infants within this group of 33 patients had a high Saling score. Fig. 4 exemplifies monitoring of the FHR and contractions during labour in this group. It will be noted that at an early stage of labour (a) the basal FHR was within the normal range, showing the normal irregularity while early deceleration occurred occasionally during the contractions. During the second stage of labour (b) rather deep early decelerations occurred with all contractions, but the basal FHR was still normal. Immediately before delivery of the head the basal FHR was slow between contractions (bradycardia). On the curve illustrating the contractions it may be seen that the patient could strain about three times during each contraction.

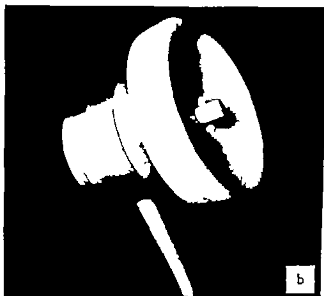


Fig 2 (*a*) The Cardiotocograph with the two-channel writer (*b*) The combined transducer with the pressure-sensitive capsule in the middle

In the Cardiotocograph microphone and pressur sensitive transducer combined capsule which is secured by a belt on the maternal abdomen where the heart sound is most distinct. The foetal heart sound is udible in a loudspeaker and is also converted to the instantaneous heart rate which is able on scal and recorded, together with the contractions, o a two-channel writer The recording is done on heat-sensiti paper which moves at a speed of 1 or 2 cm/min.

Apart from this, direct electrocardiographic recording f the FHR was employed. To this end we used the vaginal electrode designed by Hon (14, 15) in collaboration with the firm Corometrics (cf. Fig 3). This electrode is applied to the presenting part of the foetus after the membranes have been ruptured It is bipolar lead, the negative lectrode on the foetus and the positive one, at distance of about 1 cm, in contact with the mother by way of the secretions in the vagina. With silver–silver chloride electrodes and perfect isolation, this method gives a pure foetal electrocardiogram which is pplicable f recording the FHR under all conditions. The electrode, somewhat like an agraffe, is applied with special forceps, either directly r—better—through the amnioscopy tube used for taking micro blood specimens Its application requires, part from ruptured membranes, cervical dilatation of 2 3 cm

The signal is amplified pre-amplifier nd an electrocardiograph where the ECG may be recorded and from which the amplified signal is conveyed to the cardiotocograph where t may be employed instead of the phonocardiographic signal for recording the FHR.

The necessary simultaneous recording of uterine activity was done either according to the patient state ments by marking the onset and termination of the contracti on the FHR curve or by using the combined transducer of the Cardiotocograph for pure recording f the contractions.

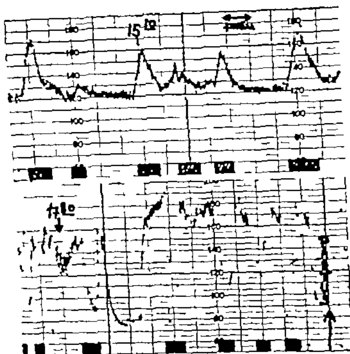


Fig. 6 (1350/68). Hypoxia resulting in severe brain damage. At 15.10 hours accelerations during the contractions, and the irregularity is on the verge of being minimal. At 17.30 hours tachycardia and very deep and protracted late deceleration.

the contractions was due to a combination of head compression and cord compression, both of which give rise to increased vagal tonus.

Hypoxia developed in two cases. In one, the FHR aroused no suspicion of a cord complication, but the monitoring failed during the last 15 min. The infant received a Saling score of 8 points and did not exhibit any abnormalities later. The other case is reported here.

Case rec. 1656/68 (Fig. 5). A gravida I whose pregnancy had been normal with normal excretion of oestriol and normal amnioscopy. At term, spontaneous rupture of the membranes occurred and the amniotic fluid was clear. Uterine stimulation was instituted with oxytocin. A vaginal electrode was applied, and monitoring was continued for 6 hours. As apparent from Fig. 5 there were periods of marked irregularity at around 18.35 hours (*A*) and 20.15 hours (*B*), indicating slight cord irritation. At 21.05 hours the pH was 7.31. About 22.50 (*C*) variable decelerations occurred, and the basal FHR had risen to upper normal limit. During the second stage of labour (*D*) there were deep early decelerations and slight late decelerations. Now the basal FHR was above the normal range but showed normal irregularity. The infant, delivered at 00.20 hours, was given Saling score of 9 points. The cord was wound once around the neck, and there was fresh meconium. The pH, measured one minute after birth, was 6.98.

In other words, this was a case of moderate hypoxia caused by compression of the cord.

Meconium-stained amniotic fluid

Within this group of 10 patients 4 had placental insufficiency asserted by the urinary excretion of oestriol. In 6 of these cases, including one with low oestriol values, there was nothing of note in the course of labour and all the infants were given 12 Saling points. In a seventh case, where Caesarean section was performed because of a low excretion of oestriol (7.8 mg/24 hours in the 41st week), the FHR curve was normal just before the operation and the infant was given a Saling score of 11. The last 3 patients will be reported in some detail below.

Case rec. 1350/68 (Fig. 6). A gravida I, not previously seen during her pregnancy, was admitted with contractions in the 35th week. When the membranes ruptured the amniotic fluid was found to be meconium-stained. A vaginal electrode was applied and the FHR monitored during the next 4 hours. Until half an hour before delivery the basal FHR was normal, but accelerations occurred in relation to the contractions, and the irregularity was at the borderline to normal. During the last half-hour the basal FHR increased to above the normal range, but throughout labour there occurred only one late deceleration, but it was very pronounced (cf. Fig. 6). On auscultation the heart sound was normal. The infant weighed 2 200 g and received a Saling score of 7. Later, it was discovered that the baby had suffered severe brain damage by perinatal asphyxia, and it died at the age of 4 months.

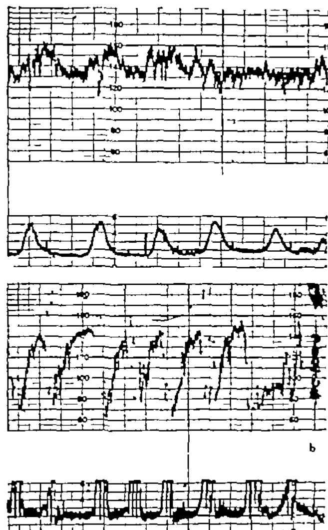


Fig 4 FHR nd contraction monitoring in normal labour A vaginal electrode is used fo the FHR recording. ( ) At an early stage of labour (*b*) The second stage f labour Early decelerations during the second stage and bradycardia immediately before deli ery f the head.

The irregularity was within the normal range in all 33 patients. The basal FHR was within the normal range in all but one in whom tachycardia was observed. During the last few minutes be fore delivery of the head, however bradycardia was a common finding (observed in about one quarter).

Early deceleration during the second stage of labour was found in about half the patients, but as a rule not as deep as seen on Fig. 4 Accelera tions during the contractions were observed oc casionally during the first stage of labour Two patients had a few late decelerations, but none had variable decelerations.

Cord around the neck

As a rule, the cord around the infant s neck is not considered a complication. The reason why this group of 15 is included here is that variable decelerations, representing compromised circula tion in the cord, might be expected to occur par ticularly in these cases. Variable decelerations were observed at an early stage of labour in 4 out of the 15 patients. In half the cases early decelerations were indeed seen during the sec ond stage of labour but deeper than in the nor mal labours. Perhaps the very slow FHR during

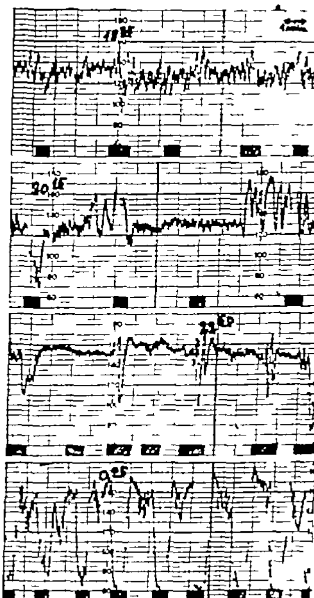


Fig 5 (1656 69). Gradually developing hypoxia due to cord compression. At *A* and *B* marked irregularity t *C* variable decelerations, and at *D* tachycardia, deep early and slight late decelerations.

Case rec. 1323/68 (Fig. 8). A gravida I with pre-eclampsia and very low oestriol values. In the 37th week the oestriol excretion was 5.7 mg/24 hours, and the foetal weight was estimated to be 1 000 g. Recording of the FHR then (11.10.1968) exhibited no abnormalities. In particular, the irregularity was normal. Spontaneous contractions occurred during the examination. Labour started in the 38th week. At first, Caesarean section was contemplated, but the idea was abandoned because the foetal weight was estimated to be 1 000 g, the amniotic fluid was thick and green, and the FHR monitoring revealed that the foetal condition was poor. The FHR was monitored for 3 hours, with vaginal electrode during the last 2. Throughout the period there was tachycardia and very deep early decelerations, followed by equally deep late decelerations (distinctly visible at 5.00 hours). Gradually the irregularity disappeared entirely. At 5.25 hours severe bradycardia appeared, and after 5.45 the FHR could no longer be recorded on the Cardiotocograph, while the electrocardiogram showed sporadic heart beats for another 20 min. A stillborn infant weighing 1 150 g was delivered at 6.46 hours. Autopsy showed severe prematurity but no malformations.

The changes in FHR occurring during labour are understandable. Already at an early stage of labour there was severe acidosis causing an increased sympathetic tonus and possibly liberation of adrenaline leading to tachycardia. During the concentrations, the intracranial pressure was increased due to the very soft cranial bones, and this resulted in deep early decelerations. Owing to the severely reduced placental function the contractions were bound to aggravate the hypoxia quite appreciably resulting in deep late decelerations. At last, there were irreparable myocardial changes with severe bradycardia, ending in total cardiac arrest.

DISCUSSION AND CONCLUSION

Our experience of abdominal FHR and contraction monitoring by the Cardiotocograph accords with that reported by others (18, 23). This investigation has the virtue of being easy to perform, devoid of risk to mother and foetus, and undisturbed by extraneous noise. It is an advantage also that the monitoring may be done before as well as after rupture of the membranes.

In our experience the applicability of the Cardiotocograph is limited. When used for some length of time it annoys most patients, as the capsule on the abdomen is rather large and has to be tightly secured in order to function. It is even more important that monitoring can in fact only be made with the patient supine, a thing which most labouring women are unable to accept for any length of time. Furthermore, the FHR curve is usually insufficient during the second stage of labour. The main use of the Cardiotocograph is therefore, in our opinion, periodic monitoring of the FHR during the first stage of labour.

The use of direct foetal electrocardiography for measuring the FHR is technically more complicated, but in return it yields more. It presupposes that the membranes have ruptured, but once the electrode has been applied the apparatus causes the patient no discomfort, and the quality of the FHR curve is excellent in all phases of labour.

We have not succeeded in solving satisfactorily the problem of the necessary simultaneous recording of the contractions. Noting them down according to the patient's statements does not afford sufficient information and is cumbersome. The use of a combined transducer for recording only the contractions is also not an ideal solution. It is true that in these cases the capsule need not be fastened so tightly to function, and the monitoring may be accomplished while the patient is lying on her side and may be continued for several hours without complaints. However the capsule is too large for this purpose, but the manufacturer is developing a smaller transducer for external recording of the contractions.

Beyond doubt the best solution is internal recording of the contractions. When a catheter is inserted into the uterus, a correct measurement of the intrauterine pressure during labour may be obtained. Hon (22) and Kubli (20), who use this combined monitoring routinely report that it has markedly reduced their perinatal mortality and the number of infants born with hypoxic damage.

Combined external and internal monitoring of the contractions and the FHR as outlined here is the best solution available at present, but the equipment is still too complicated and expensive for more extensive clinical use.

The use of the FHR curve as a parameter of foetal welfare is complicated by the numerous factors which affect the foetal heart. The numerous changes seen during labour may be difficult to interpret, and what has been interpreted as abnormal or normal does not always accord with the infant condition at birth. Although many

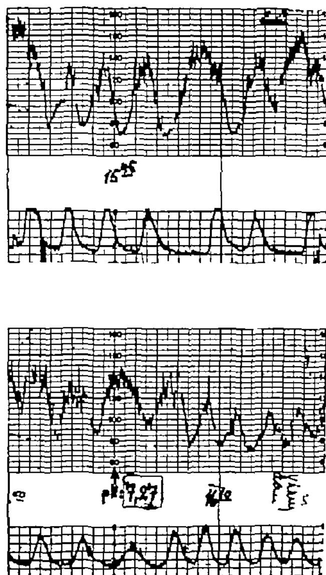


Fig 7 (437/69). Moderate hypoxia, presumably caused by excess stimulation f the contractions. Constant deep late decelerations until delivery at 16.22 hours. Bradycardia during the last 10 min before delivery

In this case the slight irregularity and perhaps the constant accelerations during the contractions represented hypoxia, but at that time the significance of the minimal irregularity had not been realized sufficiently

Case rec 437/69 (Fig 7). A gravida I with an oestriol excretion of 6.2 mg/24 hours in the 38th week and 16.5 mg/24 hours during the 40th week At term the membranes ruptured spontaneously *Meconium-stained* amniotic fluid. M nitoring was done for 2 / hours, during the last 1 / hours w th aginal lectrode. At the outset, the basal FHR was normal At hour 14 00 an oxytocin drip was instituted. The pH was 7.36 at 14 40 hours. At 15.‥5 late decelerations started t appear after each contraction and continued until delivery at 16.22 hours. The pH was measured again at 16.05 hours, th time it was 7.27 The baby was given a Saling score o 11 points and has not later exhibited any abnormality

After the marked late decelerations in this cas the infant might be expected to be depressed but the pH at birth was normal, though some what decreasing. Perhaps the late deceleration were due to an excess stimulation of the uterine activity

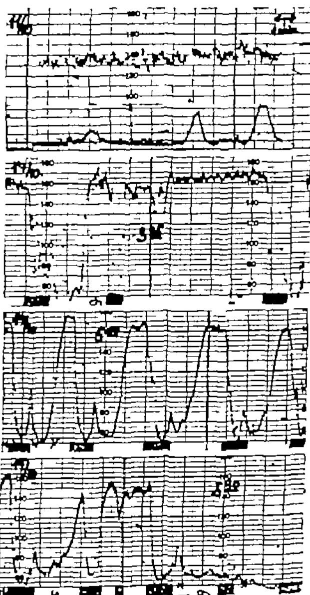


Fig 8 (1323/68). Severe placental dysfunction A premature infant *f 1 150 g who died during labour One* week prior to delivery (11 10.1968) the FHR curves had been normal, also during contractions. Du ng labour there was tachycardia, minimal rregularity and deep decelerations replaced by deep late decelerations. At 05 30 hours severe bradycardia, and death followed few minutes later *Delivery at 06 45 hours*

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LUTEAL INSUFFICIENCY AND PELVIC ADHESIONS

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Abstract A young woman with a history of septic abortion and left oophorectomy for dermoid cyst was investigated before and after laparotomy with regard to the function of the corpus luteum. At laparotomy the remaining right ovary was surrounded and fixed by thick adhesions around the ovary The adhesions were removed. During two cyclic menstrual cycles before operation, low plasma levels of progesterone were found during the luteal phase. After the removal of the adhesions normal plasma levels of progesterone were found. The urinary excretion of oestrogens also improved. Severe pelvic adhesions might be one cause of insufficient luteal function.

The clinical term luteal insufficiency probably includes several endocrine abnormalities yet to be described. The availability of rapid assay methods for the main ovarian hormones (Brown et al., 1968 Johansson, 1969) facilitates the search for abnormal cycles. However up to date most of the work on insufficient corpora lutea has been done on women taking contraceptive agents (Erb & Ludwig, 1965 Ludwig & Horowitz, 1969 Larsson-Cohn et al., 1970).

We wish to report a case of severe pelvic adhesions associated with poor function of the corpus luteum as judged by the urinary excretion of oestrogens and the plasma levels of progesterone.

CASE HISTORY

The patient was a married woman, aged 30 years, with menarche at the age of 14 and regular menstrual periods thereafter A therapeutic abortion (3 months gestation) was performed without complications at the age of 19 Four years later she was admitted to the hospital with septic abortion at 4 months Later the same year a dermoid cyst of the left ovary was removed and wedge resection was performed on the right ovary due to enlargement At the age of 27 the woman was readmitted for sterility pelvic adhesions and enlargement of the remaining right ovary At operation extensive pelvic adhesions were found, involving both the tubes and the right ovary The tubes and the ovary were freed from the adhesions and a wedge resection was performed on the ovary One year later the adhesions had reappeared and hormonal estimations showed signs of luteal insufficiency Therefore, a new abdominal operation was done. This time the pelvic adhesions were even more extensive than at the previous laparotomy The right ovary was surrounded and fixed by thick adhesions almost forming a capsule around it. The ovary was freed from the adhesions and fixed a few cm higher up in the pelvis.

HORMONAL FINDINGS

Four menstrual cycles were investigated, two before and two after the last laparotomy by the excretion of total oestrogens measured by the method of Brown et al. (1968) and by the plasma levels of progesterone measured by the method of Johansson (1969).

In the cycle collected 3 months before surgery (Fig. 1) a normal mid-cycle peak of total urinary oestrogens was found but no distinct luteal rise could be observed. The mid-cycle peak of luteinizing hormone, as measured by Wide (Wide & Porath, 1966), was found to be normal. The levels of plasma progesterone rose very slowly after the mid-cycle peak of total oestrogens and did not reach the high levels found in normal cycles (Johansson, 1969). The length of the luteal phase was normal. This observation was confirmed in the next preoperative cycle.

Four months after the laparotomy another cycle was investigated (Fig. 1). The urinary excretion of total oestrogens was found to be within the normal limits determined in this laboratory The levels of plasma progesterone rose steadily after

factors still remain to be elucidated we must not get such keen watchers of phenomena that we forget to take action. As Davis & McKeown (6) put it. "The obstetrician must be careful that watchful expectancy does not become the prelude to disaster"

It may be concluded that the bradycardia which may occur during the last minutes before delivery is usually of no significance Cord around the neck often causes deep early decelerations during the period of expulsion and in some cases variable decelerations at an earlier stage. Late decelerations usually represent hypoxia, especially when deep and combined with tachycardia. *The most serious sign that the foetus life is threatened is cessation of the irregularity on the FHR curve*

If FHR abnormalities are taken to mean tachycardia, minimal irregularity late decelerations, and variable decelerations, there were FHR abnormalities in 14 out of the present 86 labours. Of these 14 infants 9 were unaffected at birth, while 5 were depressed or dead. The FHR curve was normal in 72 labours, and within this group 71 infants were unaffected and only one depressed at birth. In this case the monitoring failed during the last 15 min before delivery

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CARCINOMA OF THE OVARY AND ENDOMETRIOSIS

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Abstract. The occurrence of ovarian endometriosis has been studied in 990 cases of primary epithelial carcinoma of the ovary In 357 serous and 155 undifferentiated lesions no example of endometriosis was found and only one example in 203 mucinous tumours. In the 205 cases of endometrioid carcinomas the incidence of endometriosis is 9.3% In the 36 cases of clear cell carcinoma and the 23 cases of mesonephroid carcinoma with tubular pattern the incidence was significantly higher, 19.4% and 25.9% respectively The incidence in endometrioid tumours was significantly higher in those cases in clinical stage I b as compared with other stages. Possible histogenetic relationships between endometriosis and ovarian carcinoma are discussed. Follow-up study of these patients clearly showed that it is of the utmost importance to attempt complete removal of all tumour tissue at the primary operation. Preoperative irradiation using super voltage technique is advocated.

The term Carcinoma of the ovary includes a heterogeneous group of tumours. In approximately 90% the malignant growth develops from the surface ("Mullerian") epithelium of the ovary Histologically these tumours may resemble carcinomas originating from the Fallopian tube (serous tumours) from the endometrium (endometrioid tumours), or from the mucin-producing cervical epithelium (mucinous tumours) Some tumours, however are so anaplastic that they can only be classified as undifferentiated ovarian carcinoma The mesonephroid tumours comprise a special group the histogenesis of which still is under debate. They may be subdivided into clear cell carcinomas and mesonephroid tumours with tubular structures, the latter sometimes resembling glomeruli. Some authors claim that only the clear cell type originates from the Mullerian epithelium (6, 10 17 19), while others are of the opinion that all mesonephroid tumours develop from remnants of mesonephros located in the ovary (13 14 1).

Endometrioid carcinomas have been described by a number of pathologists (4 12, 15 22) since Sampson's original paper in 1925 (16). At the *Stockholm Conference in 1961* Santesson proposed that these tumours should be classified as an entity separate from the serous carcinomas. The microscopic similarity between endometrioid ovarian carcinoma and endometrial corpus carcinoma is striking, but this does not necessarily indicate common histogenesis. On the other hand, while there is general agreement that serous and mucinous carcinomas may develop from benign or semimalignant ovarian adenomas of a corresponding histological pattern, the theory that endometrioid carcinoma has its benign counterpart in ovarian endometriosis is not generally accepted. According to some investigators (4 7 15 22) this may be true in the majority of cases, but others claim that endometrioid carcinoma represents a special category of serous carcinoma (12).

It is difficult to prove that an endometrioid carcinoma has developed directly from endometriosis. The ultimate proof is tumour area in which there is clear microscopic evidence of this kind of malignant transformation (5 6) Fatallah (8) succeeded in demonstrating such areas in 4 out of 52 endometrioid tumours, and a survey of the literature revealed 48 such cases described up to 1964 In 1966 Malloy (13) in series of 56 endometrioid tumours found 6 developing from endometriosis, and Gray & Barnes (9) reported a corresponding incidence of 5 out of 30 cases.

OWN SERIES

In the years 1945–64 990 patients with primary epithelial ovarian carcinoma were treated in the

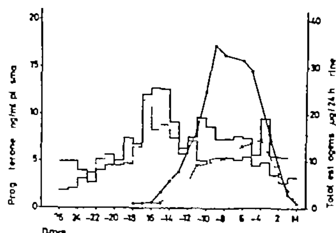


Fig 1 Total urinary oestrogens and plasma progesterone during a menstrual cycle before and after operation for severe pelvic adhesions. ---- *before operation* — *after operation.*

the mid-cycle peak of total oestrogens to reach a plateau around 15 ng/ml plasma for 5 days as in normal cycles (Johansson, 1969) This finding was confirmed in the following cycle. The length of the menstrual cycles was not affected by the surgery

The increased plasma progesterone levels after surgery is even more apparant when the values are summarized day by day Before surgery 65 4 and 58.7 ng was found as compared to 140 0 and 159 1 ng in the two cycles after surgery The sum reached before surgery is below the lowest value of 30 normal individual cycles investigated in this laboratory

It is possible that adhesions round the ovary cause the egg to remain in the follicle in spite of rupture and luteinization. Trapping of the egg has been proposed as one reason for impaired luteal function (Ludwig & Horowitz, 1969)

The case reported here suggests that local factors such as adhesions around the ovary can be responsible for insufficient function

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ble III. *Endometrioid carcinoma. Five-year survival rates in relation to the occurrence of endometriosis*

linical stage	Without endometriosis: No. of patients	Observation time too short at interval	Dead from cancer	Alive	Cancer survival rate (life table technique), %	With endometriosis: No. of patients	Observation time too short at interval	Dead from cancer	Alive	Cancer survival rate (life table technique), %
	66	12	23	51	71.3	12	0	5	7	58.4
I	45	2	27	16	38.6	4	0	4	2	33.3
II	42	1	38	3	8.5	0				
V	13	0	0	0	0.0	1	0	1	0	0.0
Total	166	15	88	70	50.7	19	0	10	0	47.4

respectively 5 and 6 years after primary treatment.

In the series of 205 cases of endometrioid carcinoma the incidence of endometriosis was 9.3% (*Table II*). Endometriosis was most frequently found in the clinical stage I b (tumour located in both ovaries), and the difference between the incidence rate of 29.4% in this stage and that of the other clinical stages was statistically significant ($p < 0.005$). The age distribution of the patients is shown in Fig. 1. In the age group 30–39 years endometriosis was observed in 29.4% of the cases as compared with between 5 and 10% in all other age groups.

The prognosis for the patients with endometrioid carcinoma and concomitant endometriosis was neither better nor worse than that of the rest of the series. The observed difference in stage I lesions was not statistically significant (Table III). It should be pointed out, however that the follow-up study clearly demonstrated that it is of the utmost importance to attempt complete removal of all macroscopic tumour tissue during the primary operation. As shown in Fig. 2, survival rate for patients with stage II lesions with no visible remnants of cancer was equal to that of stage I cases, and far better than for patients who did not have a radical operation. The high frequency of endometriosis in connection with mesonephroid tumours both of the clear cell and the tubular pattern type was very striking (Table IV). In contrast to endometrioid carcinoma very few cases of bilateral tumours were found in clinical stage I. The incidence of

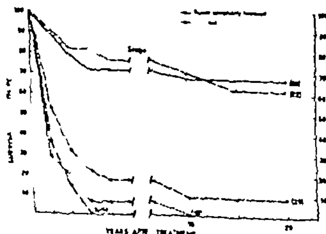


Fig. 2. Endometrioid carcinoma. Survival rate (life table technique) in relation to stage and operability. Number of patients in brackets.

Table I *Distribution by tumour type and the occurrence of endometriosis*

	No of cases	With endometriosis No.	With endometriosis %
Serous cystadenomas, low potential malignancy	74	0	0.0
Serous carcinomas	283	0	0.0
Mucinous cystadenomas, low potential malignancy	80	0	0.0
Mucinous carcinomas	123	1	0.8
Endometrioid tumours, low potential malignancy	7	1	14.3
Endometrioid carcinomas	205	19	9.3
Mesonephroid tumours of clear cell type	36	7	19.4
Mesonephroid tumours with tubular pattern	23	7	30.4
Undifferentiated carcinomas	155	0	0.0
Sinus endodermal tumours	4	0	0.0
Total	990	35	3.5

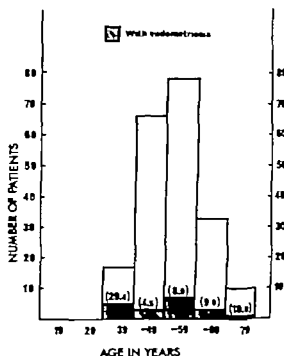


Fig 1 Endometrioid carcinoma. Age distribution and occurrence of endometriosis in the different age groups. Percentage of patients with endometriosis in brackets.

Norwegian Radium Hospital. In 1969 all the original microscopic slides were reviewed and classified according to the rules adopted by FIGO. During this review special attention was paid to the occurrence of endometriosis. All patients have been followed-up for a period varying between 5 and 20 years. The results of treatment in relation to histological type and clinical stage have been published elsewhere (1, 2, 3). In the present paper some observations will be discussed relating to the occurrence of endometriosis in the endometrioid and mesonephroid groups of ovarian carcinoma particularly.

Of the total number of 990 cases, 161 were classified as potentially malignant lesions, 74 of which were serous, 80 mucinous and 7 endometrioid tumours. The true invasive carcinomas comprised 829 cases, of which 283 were serous, 123 mucinous, 205 endometrioid, 59 mesonephroid, and 155 undifferentiated tumours. The mesonephroid group included 36 clear cell and 23 tubular pattern carcinomas. In addition 4 cases were classified as Teilum's sinus endodermal tumour.

As shown in Table I endometriosis was, almost without exception, only found in connection with endometrioid and mesonephroid carcinomas. One single case of endometriosis was revealed amongst a total of 203 mucinous tumours, and none in the groups of serous and undifferentiated lesions. However, since the study is retrospective, the observed incidence rates listed in the table must be considered as minimum figures. Seven endometrioid tumours were classified as potentially malignant lesions, six of which belonged to the clinical stage I. In one of these was found concomitant ovarian endometriosis. One patient with a stage I and one with a stage II b lesion died

Table II *Endometrioid carcinoma. Relation between stage and endometriosis*

Clinical stage	No. of patients		Per cent		Cases with endometriosis No. of patients		Cases with endometriosis Per cent	
I a	63		30.7		5		7.9	
I b	17		8.3		5		29.4	
I	18	*98*	8.8	*47.8*		*12*	11.1	*12.2*
II a	14		6.8				14.3	
II b	37	*51*	18.0	*24.8*	4	*6*	10.8	*11.8*
III		*42*		*20.5*		*0*	0.0	*0.0*
IV		*14*		*6.8*		*1*		*7.1*
Total		205		99.9		19		9.3

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Table IV Mesonephroid carcinoma Relation between stage and endometriosis

Clinical stage	Clear cell carcinoma No. of patients	Clear cell carcinoma With endometriosis	Mesonephroid carcinoma with tubular pattern No of patients	Mesonephroid carcinoma with tubular pattern With endometriosis	Total No.	With endometriosis No.	With endometriosis %
I a	20	6	11	4			
I b	1	1	0	0			
I c	1	0	0	0	33	11	33.3
II a	3	0	0	0			
II b	7	0	6	2	16	2	12.5
III	1	0	3	0	4	0	0.0
IV	3	0	3	1	6	1	16.7
Total	36	7 (19.4)	23	7 (30.4%)	59	14	23.7

endometriosis, however was much higher in this stage than in the more advanced lesions with cancer spread outside the ovaries. The 5 year survival rate in stage I for patients with clear cell carcinoma with or without endometriosis was 69.2 and 70.4% respectively The corresponding figures for the tubular pattern group were 50.0 and 84.7% but the observed difference was not statistically significant.

COMMENTS

The present investigation is retrospective. In a prospective study the incidence rates for endometriosis probably would have been found greater since more sections could have been taken from different areas of the tumours. However the observed incidence of 9.3 per cent in the group of endometrioid carcinoma corresponds well with other reports in the literature (8, 9 13). It is noteworthy that endometriosis was found more often in stage I b than in the other clinical stages, an observation which may support the theory that endometrioid tumours develop from endometriosis. In the more advanced stages overgrowth of carcinomatous tissue possibly may have destroyed adjacent endometriotic plaques.

The high frequency of endometriosis in the series of mesonephroid carcinoma is striking. 23.7% but is in agreement with earlier studies (5 6, 17 19) It has been suggested that this is an argument in favour of the theory that there exists a histogenetic connection between endometrioid and mesonephroid carcinomas, and that it casts doubt on the hypothesis that clear cell carcinoma and mesonephroid carcinoma with tubular structures develop, respectively from the Müllerian epithelium and from remnants of mesonephros (6, 10 11 17). In the present study the percentage of endometriosis in the tubular pattern group of lesions was higher than that of the clear cell carcinoma, but the difference was not statistically significant.

The occurrence of endometriosis did not seem to influence the prognosis. Of great importance, however is the radical nature of the primary operation. In our experience preoperative irradiation with supervoltage machines has greatly facilitated the dissection of the tumour Not infrequently patients who have been referred to our hospital because of ovarian carcinoma deemed inoperable at laparotomy may have their malignant growth completely removed two to three weeks after completion of external betatron 31 MeV or Cobalt60 irradiation. We prefer a midpelvis dose of approximately 3 000 rad. The gynaecological oncologist of course must be well trained in intestinal and urological surgery for in ovarian cancer an aggressive surgical approach is necessary if optimal results are to be achieved.

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MASS SCREENING FOR CANCER OF THE UTERINE CERVIX IN ØSTFOLD COUNTY NORWAY

An Experiment

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Abstract Periodic mass screening for cervical cancer has been carried out in defined population in Norway since 1959 the main objective being to evaluate various aspects of such a programme. The study population consists of 46 500 women, 25–59 years of age at the start of the programme. Attendance has been falling at successive screenings. Among those who never attended, the incidence of cervical cancer is high and the disease tends to be diagnosed at late stage. Cases detected at the periodic screening are generally in an early stage and the tumours are usually of relatively low-grade malignancy. In contrast, cases diagnosed between screenings in women who have attended at least once, are more advanced and the majority are poorly differentiated tumours. The mortality rate in this group is high. The stage distribution of invasive cases in the total study population has improved substantially whereas the effect of the programme on the incidence of the disease so far has been less striking.

In 1959 the Norwegian Cancer Society started cytological mass screening project in the County of Østfold, Norw y. Objectives, methodology and the results of the first and second screenings are described in detail in Supplement 11 to this Journal (1). The purpo of the present paper is to gi a brief summary of the main results of the second screening, to compare some of the results with those of the first screeni g, and to assess the cervical cancer situation in the county during the study period 1959–68, 1968 being the latest year for which complete data are available.

The study population consists of 46 500 women 25–59 years of age at the start of the programme. The original plan w to screen as many as possible of the eligible women at least three times, with an interval of years between the first and second screening, and 3 years between the second and third screening. It has now been decided to carry out a fourth screening approximately 3 years after the third round.

Attendanc

Both among the married and the unmarried the attendance rate at the second screening (69.6% and 42.3%) was lower than at the first (79.8% and 50.0%). However by the end of the second screening approximately 83% of the eligible population had been examined at least once.

Detection rates

I accordance with many other mass screening projects, the observed incidence rates of both in situ and invasive cervical cancer detected at the second screening were lower than those of the first screening (Table I). It is likely that the greater part of this reduction is due to numbe of preinvasive lesions and frankly invasive case being detected at the f rst examination and therefore not appearing in the subsequent data. Th stage distribution of the cases detected was favourable and confirms the general impression tha cases revealed primarily on the basis of cyto logical screening test tend to be very early

Total cervical cancer situation in the county

T get a complete picture of the effect of screening programme in g en population, it is necessary to follow not only those attendi g the

Table I. *Mass screening in Østfold, Norway Cases of in situ and invasive cervical cancer detected at first and second screening*

	No of women screened	Invasive cases: Numbers	Invasive cases: Per 1000 screened	In situ carcinoma: Numbers	In situ carcinoma: Per 1000 screened
First screening	35 528	46	1.3	146	4.1
Second screening	31 109	23	0.7	69	2.2
Previously screened	28 144	18	0.6	54	1.9
Not screened before	2 965	5	1.7	15	5.1

screening, but also the cases of cervical cancer being detected outside the programme. In Table II is shown all invasive carcinomas of the cervix diagnosed in Østfold 1964–68 among resident women eligible for screening, by clinical stage at diagnosis, according to whether or not the cases were detected by the screening programme and whether or not the women had previously been screened. It is recalled that the programme started in 1959. In 1964 every municipality in the county had been covered at least once by the screening and more than 80 % of the ever-married women had attended at least once. By the end of 1968 the third screening was nearly completed. The coverage was thus fairly good, by any standard.

Table II reveals a striking difference in stage distribution between those "Detected at screening" and those "Diagnosed at conventional consultation". Another striking observation is that no less than 27 out of a total of 95 cases, and the majority in Stage II or higher, stem from the relatively small proportion of women who never attended the screening. The size of this subgroup is continually changing, but is estimated to be approximately 17 % by the end of 1966. Based on this percentage the average annual incidence of invasive cervical cancer among those never screened can be estimated to be approximately 70 per 100 000 or twice the rate for the total female population of corresponding ages during the years preceding the screening. These data thus confirm the common experience that those women who fail to participate in screening programmes are indeed high risk groups.

The 23 patients who had previously been screened with negative results, but nevertheless had carcinoma diagnosed at subsequent conventional consultation, also show a more unfavourable stage distribution than those in whom the disease was detected by the screening programme. For the majority of these women the time for the next test had not yet come when they noticed symptoms that induced them to consult a doctor. Review of the biopsy material revealed that as many as 20 of these 23 cases had poorly differentiated squamous cell carcinomas, one was classified as a highly differentiated squamous cell

Table II *Mass screening in Østfold, Norway New invasive cases of cervical cancer diagnosed 1964–68 among resident women eligible for screening (25–59 years of age at start of programme), by clinical stage at diagnosis, according to whether the cases were detected by the screening programme and according to whether they had previously been screened*

	I A	I B	II A	II B	III	Total
Detected at screening						
Previously screened	22	7		1		30
Not screened before	7	8				15
Diagnosed at conventional consultation						
Previously screened	1	11	7	4		23
Never screened		*10*	*4*	*7*	*6*	*27*
Total	30	36	11	12	6	95

carcinoma and two as highly differentiated adeno-carcinomas.

Table II thus illustrates some important features which periodic cytological mass screening programmes have in common with other types of periodic mass examinations: a tendency to miss the high-risk groups, a tendency to detect selectively the more benign, slowly developing cases, and a tendency to overlook a certain proportion of the cases among those attending, which often are of a high-grade malignancy. This explains why mass screening projects in many parts of the world thus far have had only a modest effect upon the mortality rates of cervical cancer.

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PROGNOSTIC VALUE OF QUANTITATIVE DETERMINATION OF RHESUS ANTIBODY (ANTI D) IN MATERNAL SERUM AND AMNIOTIC FLUID

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Abstract. Quantitative determinations of anti-D in sera and amniotic fluids were performed simultaneously in 63 pregnant Rh-immunized women with Rh positive foetuses. I was demonstrated that the ratio of serum antibody to amniotic fluid antibody as a rule is the same.

A quantitative analysis of the serum antibody enabled us to identify the foetuses with good prognoses. For the remaining cases, it was possible to distinguish between moderate to severe cases and very severe cases of erythroblastosis by determining also the concentration or titre of amniotic fluid antibody. Where specimens were taken less than a fortnight before birth, the titre values alone made it possible to prognosticate correctly in 80% cases. Correlation of amniotic fluid antibody and bilirubin levels led to correct prognoses in 93% of the cases.

With this background a plan for the control of pregnant Rh-immunized women is suggested.

The evaluation during pregnancy of the degree of severity of erythroblastosis foetalis due to Rhesus immunization can be made on the basis of (*a*) data concerning the degree of severity of erythroblastosis in previous pregnancies, (*b*) the father's Rhesus genotype (*c*) the titre of anti-D in the maternal serum, and (*d*) the amniotic fluid content of blood pigments and anti-D. An evaluation based on any one of these parameters is subject to considerable uncertainty. The anamnestic data give the least degree of certainty and determination of the amniotic fluid content of blood pigments results in the most reliable evaluation. There are several reasons why the degree of severity cannot be predicted directly from the serum titre, e.g. only the IgG component of anti-D is placenta-permeable placenta permeability for antibodies may be individual, the susceptibility of the foetuses may vary.

As first suggested and investigated by Hoffbauer (7, 8, 9) the determination of anti-D in amniotic fluid should give a better impression of the prognosis, since only the placenta-permeable component of anti-D is measured. This method has since been used by others (1, 4, 12, 16, 17, 18) with varying results.

We have examined whether simultaneous determination of the anti-D titre both in serum and amniotic fluid yields information of prognostic value.

MATERIAL AND METHODS

The series consists of 70 pregnant anti-D immunized women, on whom amniocentesis was performed once or several times during pregnancy. Sixty-three of these 70 mothers bore Rh positive Rh-sensitized infants. Amniocenteses were performed on 50 of these 63 mothers less than a fortnight before delivery.

Specimens of amniotic fluid and serum were sampled on the same day and examined for anti-D content. Amniotic puncture was performed at the earliest in the 28th, and at the latest in the 40th week of pregnancy. The examination was carried out as double dilution titration using the indirect Coombs technique and the 2-step papain technique by the method of Goldsmith (6). For all titre determinations erythrocytes from the same person with Rhesus genotype CDe/cDE were used. The amniotic fluids were also tested for their content of unconjugated bilirubin according to Brodersen's method (3).

To evaluate their prognostic value, the antibody titres were related to the concentrations of haemoglobin in the cord blood, this latter value being considered the best single criterion for predicting the severity of erythroblastosis (15).

RESULTS

The 2-step papain technique demonstrated the presence of anti-D in all the examined serum

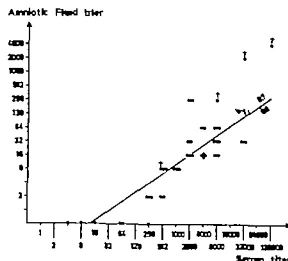


Fig 1 Titres of anti-D in serum and amniotic fluid from 63 Rh-immunized women.

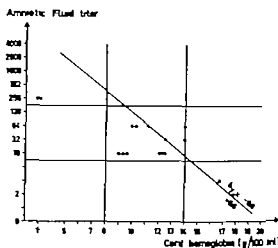


Fig 3 Titres of anti-D in amniotic fluid and haemoglobin concentration of cord blood in 38 cases where the serum titre of anti-D was higher than 256 and the mother bore a Rhesus positive child.

specimens and in 57 (91 %) of the corresponding amniotic fluid specimens.

Complete saline anti D was not found in any of the amniotic fluid specimens, although it was present in 23 % of the sera with titres ranging from 16 to 16 000 in saline.

Fig. 1 indicates the values of the anti D titres in serum and in amniotic fluid Only the values found by means of the 2-step papain technique are given It is seen that anti D was found in the amniotic fluid when the serum titre was higher than 8 The values are located close to a straight regression line having an inclination of 0 7 and a correlation coefficient (*r*) of 0 8, suggesting a good correlation between the two values.

A few of the values are, however placed quite far away from the line, which implies that there has been more or less placenta permeable anti-D than usual for a given serum titre value. On an average the serum titre is 7 serial dilutions greater than the corresponding amniotic fluid titre When the difference between serum titre and amniotic fluid titre proved to be less than 7 dilutions, more antibody than usual must have passed into the amniotic fluid. Other things being equal, this might imply a less favourable prognosis for the foetus than is usually the case for the serum titre in question. Perinatal death occurred in 5 out of the 50 cases where amniotic puncture was performed less than a fortnight before birth The foetuses were still alive when the amniocenteses were performed. In these 5 cases the difference between the serum titres and the amniotic fluid titres were respectively 1 2, 2, 3 and 4 dilutions less than average In *Fig. 1* the 5 cases are marked †

Fig. 2 shows the value of the serum titre and of the haemoglobin content of the cord blood in the 50 cases, where the specimens were taken less than a fortnight before birth. As previously demonstrated by Mollison & Cutbush (14) and by Freiesleben (5) there is a relatively good correlation between the two values ($r = 0\ 6$) In order to relate the cord haemoglobin values to the clinical course of events, dividing lines have been drawn

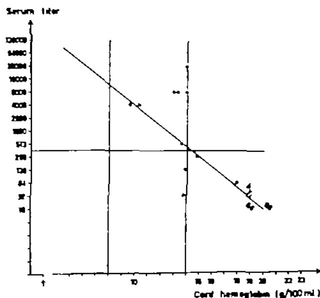


Fig 2 Titres of anti-D in serum and haemoglobin concentration of cord blood in 50 cases, where Rhesus positive child was delivered less than 14 days after amniocentesis.

at the haemoglobin values 8 g% and 14 g%. Infants having a cord haemoglobin level of more than 14 g% (risk group I) are usually unaffected and hardly ever need exchange transfusion, whereas infants with a cord haemoglobin level below 8 g% (risk group III) are always severely ill, presenting a risk of neonatal death. I fants in the intermediate group (risk group II) are as rule moderately affected by erythroblastosis, requiring therapeutic intervention. Fig. 2 shows that in 12 out of the 50 cases the serum titres were 256 or below. In 3 of these 1 babies cord haemoglobin alues were less than 14 g% (13.8, 13.9 and 11 7 respectively): the other 9 belonged to risk group I. The remaining 38 cases in which the serum antibody titres were above 256 were distributed in such a way that they could not be divided into risk groups on the basis of the serum titre, whereas the amniotic fluid titres made such a division possible. In Fig. 3 the amniotic fluid titres for these 38 cases are related to the observed cord haemoglobin values. A good correlation was found between the two values ($r = 0.6$). A division f the 38 cases into three risk groups, drawing dividing lines at the amniotic fluid titres between 8 and 16 and between 128 and 256, fits well the division based on the cord haemoglobin values, except in 7 cases.

DISCUSSION

Estimation of the prognosis for Rh-positive foetuses of Rh-immunized mothers based on the amniotic fluid content of anti-D has previously been made by Hoffbauer (7 8 9), who examined the amniotic fluid from 139 Rh-immunized pregnant women. Using the -step papain technique he found anti-D in 40 of the cases, and 50 *of the babies in this group died* in the other 60% where he did not find anti-D in the amniotic fluid, the babies survived without therapeutic intervention. Beecham et al. (1) and David et al. (4) examined amniotic fluid specimens from 8 and 25 pregnant women respectively applying the indirect Coombs technique: they found no relation between the qualitative or quantitative presence of anti D and the prognosis for the foetus.

Using the papain technique Kubli & Murak (1) examined the amniotic fluids of 19 pregnant Rh-immunized women, and observed that the presence of Rh-antibody suggested poor prognosis; on the other hand, the absence of Rh-antibody in the amniotic fluid did not exclude poor prognosis.

By means of the albumin-papain technique, Bierme et al. (2) demonstrated the presence of anti-D in the amniotic fluid of 57% of 102 anti-D immunized pregnant women. They found that the presence of anti-D in amniotic fluid implied an unfavourable prognosis, and that an anti-D titre higher than 8 in amniotic fluid was a better index of an unfavourable prognosis than a serum titre higher than 1 000.

Murray (16) examined 203 amniotic fluid specimens from 126 Rh-immunized pregnant women, finding Rh-antibodies in 38% of the specimens by means of the papain technique. Out of the cases having serum titres lower than 64 (indirect Coombs technique), 23% had Rh-antibody in the amniotic fluid, and of those having serum titres of 64 or above, 57% had Rh-antibody in the amniotic fluid ($p < 0.005$). It was ascertained that whenever antibodies were found in the amniotic fluid of women, who had previously born erythroblastotic infants, the cord haemoglobin values were considerably lower ($p < 0.02$), the serum bilirubin values higher ($p < 0.01$), and the number of stillborn children was greater ($p = 0.0002$).

Using the indirect Coombs technique, Usategui-Gomez et al. (18) examined the amniotic fluid content of Rh-antibody in 4? Rh-immunized mothers, and on this basis could place 84% of the infants in the correct risk groups. An examination of the bilirubin in the amniotic fluids enabled them to place 6_% of the same infants correctly ($p < 0.01$). Using both methods, they arrived at 95% correct predictions.

We found anti-D in the amniotic fluid only when the serum titre exceeded 8, and we found marked correlation between the amount of anti-D in serum and the amount of anti-D in amniotic fluid, their corresponding values being close to straight line having correlation coefficient of 0.8. However it may occur that there is more anti-D in amniotic fluid than is usually found for the corresponding serum titre. In such cases one would expect the prognosis to be worse than usual for that particular serum titre. In Fig. 1 the points indicating perinatal deaths (4 intrauterine deaths and 1 neonatal death, marked †) are, in fact, to be found abov the upper right-hand part of the curve.

It appears from Fig 2 that a serum titre of 256 or less indicates little risk for the foetus, all the babies except one having cord haemoglobin values of about *14 g%* or more. It was not possible to make a division into risk groups on serum titres higher than 256 and in such cases the division was made according to the amniotic fluid titres instead Fig. 3 shows that if all cases having amniotic fluid titres below 16 are placed in group *I* all cases having *amniotic fluid* titres ranging from more than 8 to 128 inclusive are placed in group II and the remaining cases in group III 31 (82%) of the cases having serum titres higher than 256 will be found in the correct risk *group* If we include the cases in which the serum titres were *256 or below correct allocation into risk* groups was possible in 40 (80%) of all 50 cases.

Amniocentesis increases the risk of foeto-maternal bleeding (10 11 13 17 19) Therefore to avoid an additional immunization stimulus, it is inadvisable to perform amniotic puncture when the risk for the foetus is low

A determination of both antibody and bilirubin content in the amniotic fluid is, however of great value if the serum titre and the anamnesis indicates an increased risk for the foetus. The antibody as well as the bilirubin content of the amniotic fluid gives more reliable information about the condition of the foetus than does the antibody content of the serum. Furthermore, it is preferable to use both of these independent parameters in predicting the outcome of pregnancy as probably they do not both fail in the same instances.

A single determination of the amniotic fluid content of unconjugated bilirubin made it possible to place 27 (71%) of the 38 cases having serum titres higher than 256 in the correct risk group Repeated determinations of the amniotic fluid bilirubin level will, however greatly enhance the reliability of the prognosis. Out of the 7 cases where the amniotic fluid titres of anti-D did not permit placement in the correct risk group it was possible to make a correct assessment of 3 by means of the unconjugated bilirubin found in the amniotic fluid. Thus a total of 46 (92%) were correctly predicted when using both parameters.

CONCLUSION

On the basis of the above-mentioned results we believe that the control of pregnant Rh-immunized women can be carried out in the following way Determination of the serum content of anti-D should be made at least once a month. If, according to the technique applied by us, the serum titre is higher than 256 or is found to increase, rising to more than 256 (a value which should obviously be determined by each laboratory), amniocentesis should be performed and the amount of antibody and blood pigment contained in the amniotic fluid should be determined. If these values place the foetus in the highest risk group the choice between induction of labour or intrauterine transfusion will depend on the size of the foetus.

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STUDIES ON DIAMINE OXIDASE ACTIVITY DURING PREGNANCY

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Abstract. The diamine oxidase activities (diamine: oxygen oxidoreductase (deaminating), (EC 1.4.3.6)) of amniotic fluid, maternal venous plasma and fetal plasma were measured in 19 pregnant women at term. No statistically significant difference was found between the enzyme activity of the amniotic fluid and the maternal plasma. The enzyme activity in the fetal circulation was approximately one hundredth of that in the other compartments. It is suggested that passage of fluid through the amniotic membrane might be of greater importance in the formation of the amniotic fluid than believed earlier. Twenty-seven women in the second trimester of pregnancy were also investigated, maternal venous blood and amniotic fluid being sampled. The diamine oxidase activity of the amniotic fluid was significantly lower than that of the maternal plasma. It is suggested that the mechanisms governing the production of amniotic fluid might vary in importance at different periods of gestation. Following intramuscular injection of aminoguanidine, a diamine oxidase inhibitor, in 10 pregnant women significantly lower enzyme activity was found in both maternal plasma and in amniotic fluid than in nontreated subjects.

In the course of human pregnancy enzymes in blood plasma which inactivate histamine increase in activity. This inactivation is caused mainly by oxidative deamination catalyzed by the enzyme diamine oxidase (diamine: oxygen oxidoreductase (deaminating) (EC 1.4.3.6)) (Lindahl, 1960). Lindahl (1960) showed that placental tissue also contains a histamine-methylating enzyme (S-adenosylmethionine: histamine N-methyltransferase (EC 2.1.1.8)) and Lindberg & Törnqvist (1966) showed an increased methylating activity in plasma during pregnancy after inhibition of diamine oxidase activity in vivo.

Although the variation of histamine inactivating activity of maternal plasma has been thoroughly investigated during pregnancy comparatively few studies on this activity in amniotic fluid and in the fetal circulation have been published. By means of a biological method Swanberg (1950) showed that the histamine inactivating activity of maternal plasma was higher than that of amniotic fluid, in early pregnancy. Southren et al. (1965) found at term a higher diamine oxidase activity in amniotic fluid than in maternal plasma. In the same investigation they found a very low diamine oxidase activity in the fetal circulation.

In the present investigation the diamine oxidase activity of maternal plasma and of amniotic fluid was measured in the second trimester of pregnancy and at term. The fetal diamine oxidase activity was measured at delivery. The effect of aminoguanidine, a diamine oxidase inhibitor, on the enzyme activity of maternal plasma and amniotic fluid was studied in vivo in the second trimester of pregnancy.

MATERIAL AND METHODS

Nineteen pregnant women at term were investigated. All pregnancies were normal. Amniotic fluid was taken at caesarean section or at amniotomy through an amnioscope. Simultaneously maternal blood was withdrawn from a cubital vein. Fetal blood was obtained from the umbilical vein immediately after delivery.

Twenty-seven pregnant women admitted to the hospital for legal abortion were also investigated. They were in the 15th to the 24th week of pregnancy. Amniotic fluid was withdrawn with a syringe immediately before intra-amniotic administration of 20% sodium chloride solution for induction of legal abortion. Maternal venous blood was withdrawn simultaneously from a cubital vein into heparinized tubes. In 10 cases in this group the effect of a diamine oxidase inhibitor (aminoguanidine sulphate) was studied. Maternal venous blood was first sampled, and intramuscular injections of 0.2 mg of aminoguanidine sulphate per kg of body weight were then given. One hour after the injection of the enzyme inhibitor samples of maternal venous blood and of amniotic fluid were withdrawn at the same time. For ethical

Table I. *Diamine oxidase activity in maternal and fetal plasma and in amniotic fluid at term*

The diamine oxidase activity in units per litre and minute

	Plasma		
	Maternal (U/l)[a]	Fetal (U/l)	Amniotic fluid (U/l)
1	0.77	0 006	0.61
2	1.30	0.004	0.27
3	1.26	0 005	0.11
4	0.68	0.007	0.22
5	0.17	0.0002	0.32
6	0.55	0 002	0 66
7	0.93	0.042	1.32
8	0 42	0.002	1.22
9	0.29	0 001	0.59
10	0.37	0 003	0.67
11	1 15	0.019	4.39
12	1 17	0.003	0.45
13	0.30	0 0001	0.94
14	0.78	0.004	1 15
15	0.44	0.004	0.34
16	1 40	0 005	1 46
17	0.34	0.004	0.44
18	0.55	—	0.53
19	0.52	0.050	0 62
Mean	0.71	0.009	0.86

One unit (U) in 1 μmole of substrate degraded per min at 37 C in air

reasons amniotic fluid was only sampled on on_ occasion in each patient, i.e. when puncture of the amniotic sac was necessary for induction of legal abortion.

All samples of blood and amniotic fluid were immediately placed at +4°C and then centrifuged within an hour Plasma was separated and stored t – 0°C. The analyses were performed within 14 days by means of a radioassay procedure originally described by Okuyama & Kobayashi (1961) and modified by Tryding (1956) The enzyme acti ity is given in units per litre one unit being defined as the amount f enzyme catalyzing the break down of one μmole of substrate per minute at 37°C in air

STATISTICS

The difference between the enzyme concentrations in amniotic fluid and maternal plasma at term were analyzed for significance with the Wilcoxon rank test for pair difference. The same test was used to examine the difference between maternal plasma and amniotic fluid in the abortion group. The difference in diamine oxidase concentration of amniotic fluid with and without enzyme inhibi tion was analyzed for statistical significance by the Wilcoxon two-sample rank test (Brownlee, 1961)

RESULTS

The figures for diamine oxidase concentrations in amniotic fluid, maternal venous plasma and fetal plasma at term are given in Table I. The individual variations in enzyme concentration are considerable in both amniotic fluid and in maternal plasma. According to a ranking test for pair differences there is no statistically significant difference between the enzyme activities in amniotic fluid and in maternal plasma. The diamine oxidase activity in the fetal circulation was in all cases much lower than that in the other two compartments.

Table II shows the diamine oxidase activity in maternal plasma in two samples drawn with a one-hour interval and in amniotic fluid of women in the second trimester of pregnancy It is evident that there is no significant difference in enzyme activity betwene the two venous samples in the same subject. However according to a ranking test for pair differences the values for amniotic fluid are significantly lower than those for venous plasma.

Table III shows the diamine oxidase activities of maternal venous plasma before and after ad ministration of aminoguanidine sulphate and of

Table II *Diamine oxidase activity in maternal plasma and amniotic fluid in the second trimester of pregnancy*

The first and second samples of maternal plasma were taken t an interval of 1 h the second sample was taken simultaneously with the amniotic sample

	Duration f pregnacy (weeks)	Maternal plasma		
		1st sample (U/l)	2nd sample (U/l)	Amniotic fluid (U l)
1	19	0.27	0 26	0.37
2	19	—	0 52	0.34
3	16	0 4	0 .5	0.09
4	17	0 31	0 32	0 19
5	17	0 15	0 17	0 05
6	17	0 46	0 42	0.20
7	24	—	0 31	0 64
8	20	0.35	0 37	0 37
9	22	0 48	0 48	0 48
10	18	0.17	0 16	0 08
11	17	—	0.27	0 10
1	22	0.48	0 49	0 4
13	1	0 65	0 71	0 15
14	17	0 21	0.20	0 06
15	17	0 .3	0 21	0 21
16	19	0 37	0 42	0 15
17	18	0 30	0.31	0.33

Table III *Diamine oxidase activity in maternal plasma before administration of 0.2 mg/kg body weight of aminoguanidine sulphate and in maternal plasma and amniotic fluid after administration of aminoguanidine sulphate*

	Duration of pregnancy (weeks)	Before administration of aminoguanidine sulphate	After administration of aminoguanidine sulphate	
		Maternal venous blood (U/l)	Maternal venous blood (U/l)	Amniotic fluid (U/l)
1	17	0.19	0.0013	0.06
2	18	0.25	0.014	0.12
3	20	0.18	0.006	0.02
4	19	0.40	0.020	0.05
5	18	0.26	0.010	0.02
6	18	0.33	0.033	0.013
7	18	0.41	0.050	0.07
8	17	0.15	0.016	0.023
9	18	0.50	0.040	0.08
10	15	0.13	0.021	0.075
Mean	18	0.28	0.022	0.051

amniotic fluid after the administration of the diamine oxidase inhibitor. These cases are from the same period of pregnancy as those described in Table II.

The enzyme concentration of the maternal venous blood before the diamine oxidase inhibition in this group of patients was compared with the figures for the group of patients presented in Table II. According to a Wilcoxon two-sample rank test there is no statistically significant difference. After enzyme inhibition the diamine oxidase concentration of the venous blood decreased in all subjects on the average 10 times. The diamine oxidase activity of the amniotic fluid after enzyme inhibition was compared with the corresponding figures given in Table II for subjects in which the enzyme was not inhibited. A Wilcoxon two-sample rank test shows significantly lower values at the 5 % level, after enzyme inhibition.

DISCUSSION

The diamine oxidase concentration in amniotic fluid in maternal venous plasma shows considerable individual variations. However repeated enzyme measurements in the same patient show that the radioassay procedure has a good reproducibility. The results of the present investigation show a different relation between the diamine oxidase activity of amniotic fluid and of maternal plasma in the second trimester of pregnancy and at term. In the second trimester the enzyme activity of amniotic fluid is significantly lower than that of maternal plasma, which is in good agreement with the results of studies reported by Swanberg (1950). In the present investigation there was no statistically significant difference between the diamine oxidase activity of amniotic fluid and of maternal plasma at term. This is at variance with the results published by Southren et al. (1965) who found the amniotic fluid to possess approximately two-to-three times the enzyme activity of the maternal plasma.

In the present investigation there was a striking difference between the diamine oxidase concentration of amniotic fluid and maternal plasma on one hand and the enzyme activity of the fetal circulation on the other, the diamine oxidase activity in the fetal circulation being at least one hundred times lower. This agrees with results published by Wicksell (1949) and Southren et al. (1965). Evidence presented by Swanberg (1950) show that the decidua is the main source of diamine oxidase during pregnancy. There is however no direct passage of enzyme as judged from the large difference in enzyme activity. This finding agrees with earlier results of Wicksell (1949) and Southren et al. (1965).

The fact that the activities of the enzyme in maternal plasma and in amniotic fluid are of similar magnitude indicates that enzyme molecules pass through the amniotic membrane. If macro-

molecules can pass through the amniotic epithelium it appears likely that also water and low molecular weight compounds pass through. It may be speculated upon that such a passage is of considerable importance for the formation of the amniotic fluid and that fetal urine and active secretion from the amniotic epithelium are quantitatively less important.

Aminoguanidine sulphate is a potent inhibitor of the diamine oxidase of human blood plasma as has been shown in several investigations, e.g. Lindell et al. (1960) Lindberg & Törnqvist (1966) Administration of this inhibitor resulted in an about five fold decrease in the enzyme activity in the amniotic fluid, whereas the decrease in enzyme activity in maternal plasma was about 10-fold.

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A PROSPECTIVE STUDY OF SMOKING AND PREGNANCY

Stig Kullander and Bengt Källén

From the Department of Gynaecology, Malmö Allmänna Sjukhus, Malmö, and the Department of Embryology, University of Lund, Lund, Sweden

Abstract. A prospective study was performed on the effect of smoking on pregnancy using information from 6 363 pregnancies with known smoking habits. Of these, 2 806 (44%) smoked during pregnancy and 2 731 (97%) of the 2 806 reported that they smoked during the whole pregnancy. The well-known effect on prematurity rate and mean birth weight was verified in this study. A 50% increase of prematurity rate was registered among smoking women compared with non-smoking women. The mean birth weight reduction was 170 g among live-born, non-malformed children. These effects were found to occur irrespective of other variables studied, maternal age, parity and whether the pregnancy was wanted. The body length, head circumference, and shoulder circumference were found to be reduced in children born to smoking women. Placental weight was also reduced, but the ratio placental weight over body weight increased with smoking. No effect on the malformation rate was observed, but the series is too small to exclude a teratogenic effect of smoking. Children were followed to 1 year of age. The death risk for a child of a smoking woman was found to be increased to 1.6 times that for a child of a non-smoking woman. No significant difference in stillbirth rate was found, but an effect was found on neo-natal death both before and after the age of 1 week, although only among non-premature children. Among children dying before the age of 1 week a significant increase in frequency of abruptio placentae was noted. No other specific cause of death was noted. An overall increased risk of spontaneous abortion among smoking women was verified, this was shown to be almost completely due to the association between the fact that the pregnancy was unwanted and smoking. An even higher incidence of smoking was seen among women with induced abortions. Spontaneous abortion was also associated with unwanted pregnancies, perhaps due to the presence of induced abortions among the group recorded as spontaneous abortions. Smoking was shown to be associated with late spontaneous abortions. If there is an abortifacient effect of smoking, it must be very slight. The decreased rate of preeclamptic complications among smoking women, described by other authors, was verified. A small decrease in frequency of morning sickness was also found. No effect of smoking on the mean Apgar score of surviving, non-malformed children was seen. The sex ratio among children born to smoking women differed slightly from that born to non-smoking women, but this difference was not statistically significant.

Since Simpson (1957) first reported on a connection between smoking habits during pregnancy and prematurity a considerable number of papers have been published on this subject, reviewed by among others, Goldstein et al. (1964), Russell et al. (1968), and Terris & Gold (1969). The mode of collection of the data has varied in different studies and different aspects of the pregnancies and their outcome have been studied. The finding of increased prematurity rate among children of smoking women has been amply verified (prematurity usually defined as birth weight below 2 500 g). This effect of smoking has been shown to exist irrespective of race, parity, maternal age, status and social class, father's age and social class, marital status, sex of child (cf. Russell et al. 1968, Pettersson, 1969). The mean birth weight reduction among children of smoking women has varied somewhat in different series from 40 g (O'Lane, 1963) to 396 g (Mulcahy et al., 1970). This is partly explained by differences in the racial and socio-economic composition of the material studied and partly by different definitions of smokers. Thus, for instance, the weight reduction was shown to be more pronounced in white women of high socio-economic standard than in coloured women of low socio-economic standard (Underwood et al., 1965).

The importance of the husband's smoking habits has been debated. MacMahon et al. (1965), Ravenholt & Levinski (1965) and Terris & Gold (1969) found no effect due to the husband's smoking habits, Underwood et al. (1967) no ef-

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Table I. *Summary of smoking habits in women studied*

Total number of women followed during pregnancy		6 376
Smoking habits not known		13
Women never smoking during pregnancy (non-smokers)		3 557
Women smoking during pregnancy (smokers)		2 806
Among the latter:	Women starting to smoke	15
	Women who stopped smoking	24
	Women who stopped smoking for some period of pregnancy	36
	Women who smoked throughout the pregnancy	2 731
Among the latter:	Smoked < 10 cigs/day	80 %
	Smoked 10–20 cigs/day	19
	Smoked > 20 cigs/day	1 %

of this problem. Duffus & MacGillivray (1968) found albuminuric preeclampsia in 2.4 % of smoking women but in 5.2 % of non-smokers. The difference could not be explained by social class, maternal weight, or maternal weight gain. In cases of severe preeclampsia, however birth weight and the length of gestation were reduced when the woman smoked, and the death rate of the children in such pregnancies was considerably increased. The explanation of these findings is not clear.

Most of these studies were retrospective, although some truly prospective studies have been performed (e.g. Frazier et al., 1961 Yerushalmy 1964 Reinke & Henderson, 1966 Russell et al., 1966, 1968). The main findings about prematurity rate and the reduction in birth weight have been made both in retrospective and prospective studies, which show that the bias of a retrospective study is small concerning the information whether or not the woman smoked during the pregnancy as was pointed out by Goldstein et al. (1964) in their review. Probably retrospective studies provide less accurate information about the amounts actually smoked during different parts of the pregnancy. Such data are best obtained in prospective studies where data are collected continually during pregnancy.

In this paper such a prospective study is presented. The main points to be discussed are the effects of smoking on the abortion rate, on child mortality prematurity and birth weight, on other body dimensions, and on placental weight.

MATERIAL AND METHODS

The material analysed in this paper is taken from prospective study on pregnancies, carried out in Malmö during the years 1963–64 (cf. Kullander & Källén, 1964). Further details of this investigation will be published separately: in this paper, only the collection of data relevant for the present study will be described. When woman came to gynaecologist in Malmö and pregnancy was diagnosed, she was asked to complete questionnaire, giving certain information about various aspects of her pregnancy. This was returned after few days, and she was handed new questionnaire to be filled in ready for the next visit to the doctor when, in turn, new form was given to her etc. In this way the whole of the pregnancy was usually covered by four questionnaires. Part of the information thus obtained was indeed retrospective in the sense that it was collected some time after the events had happened, but it was prospective to the extent that most information was collected before the outcome of the pregnancy was known.

One of the questions was, do you smoke? If so, how many cigarettes per day? As this information was collected repeatedly during the pregnancy variations in smoking habits could also be recorded. No questions were asked concerning the father' smoking habits—some mothers volunteered the information, but as this was not regularly done, it has not been used. The smoking habits

Table II. *Smoking habits of women with different pregnancy outcome*

Pregnancy outcome	Total no. of women	Number with known habits	Number smoking	Per cent smoking
Spontaneous abortion	448	445	227	51.0
Induced abortions	158	155	87	56.1
Extrauterine pregnancy	23	23	8	35
Childbirth	5 747	5 740	2 484	43.3
Among these:				
Children without malformations and living at 1 year	4 910	4 903	2 076	42.3
Malformed children, major or minor and or dead before 1 year	837	837	382	45.6

fect when the woman did not smoke, but Yerushalmy (1962) found a stronger effect on prematurity rate from the husband's smoking than from the wife's smoking and the most pronounced effect when both smoked.

In most studies, a quantitative response was found in the reduction of birth weight or the prematurity rate to the number of cigarettes smoked per day Women who used to smoke but who had stopped before the pregnancy did not differ from non-smoking women with regard to prematurity rate or child birth weight (Lowe, 1959 Yerushalmy 1962) and the length of time the woman had smoked before she became pregnant was shown to have no effect (Zabriskie, 1963) Some studies have investigated the effect of smoking during only part of the pregnancy—such women showed less reduction in birth weight (Frazier et al 1961) but smoking during any trimester affected birth weight (Underwood et al., 1967)

Mean placental weight showed no difference between smoking and non-smoking women in one study (Mulcahy et al., 1970) but Järvinen & Österlund (1963) stated that the ratio of placental weight to fetal weight was similar in both groups, indicating a lighter placenta in smoking women than in non-smoking women Järvinen & Österlund also stated that retention of the placenta was more common when the woman smoked.

The length of gestation has usually been reported to be approximately the same for smoking and non smoking women, but Buncher (1969) found a mean reduction of gestation time of 29 hours in female births and of 34 hours in male births and calculated that this shortening could only explain about 10% of the reduction in birth weight in children of smoking women. On the other hand, Järvinen & Österlund (1969) found more pregnancies of a duration longer than 41 weeks among smoking women than among non-smoking women.

In most studies, small differences in sex ratio have been found between children born to smoking and to non-smoking women usually fewer males than females were born to smoking women, and fewer females than males to non-smoking women. By adding some such series, Fraumani & Lundin (1964) demonstrated a statistically significant difference in sex ratios.

Results vary concerning the perinatal death risk associated with smoking during pregnancy Some authors (e.g., Savel et al. 1962 O'Lane 1963 Downing & Chapman, 1966 Underwood et al., 1967) found no effect on the death rate others found an increased death rate (e.g Frazier et al., 1961) or a decreased rate (e.g., Peterson et al., 1965) Yerushalmy (1964) stated that, at the same birth weight, children of smoking mothers had a better chance of survival than children of non smoking mothers. All samples of dead children were small Comstock & Lundin (1967) made a sample of 448 live births, 296 stillbirths, and 431 child deaths. Stillbirth rates for smoking and non-smoking women were found to be the same, but an increase in neonatal deaths and deaths during the first year of life was found for children of smoking women. Most of the increased hazards seemed to be associated with the low birth weight. The abortion rate among smoking women has been found to be increased in the study performed by Zabriskie (1963)—12.6% in smoking women and 8.8% in non-smoking women. Underwood et al. (1965) found a non-significant increase in the abortion rate associated with smoking, and Russell et al (1966) an increased risk of abortion, stillbirth, and neonatal death. O'Lane (1963) recorded a higher number of early abortions in smoking women than in non-smoking women. Downing & Chapman (1966) found no increase in the abortion rate among smoking women, but probably studied mainly late abortions.

Fetal distress has been said to be somewhat more common among children of smoking women than of non-smoking women (Herriot, 1962 Heron, 1962) and a reduction of the Apgar score, although not clinically important, was described by O'Lane (1963) On the contrary Peterson et al (1965) and Russell et al. (1968) found no effect of smoking on mean Apgar score.

No definite effect on the malformation rate has been demonstrated but the series studied have been small. In one study slightly more malformed babies were recorded when the mother smoked in another series, more were found among non-smoking women.

In most studies, maternal complications have been of the same frequency in smoking women as in non-smoking women. In some studies, pre-eclampsia or toxaemia was found less often in smokers than in non-smokers (e.g Underwood et al., 1967 Zabriskie 1963) In a special study

ers smoked more than 10 cigarettes per day but among women with an unwanted pregnancy 27.1% did so. This difference is statistically significant ($\chi^2 = 7.7$ at 1 d.f. $0.01 > P > 0.001$). The fraction of constant smokers among all smokers is the same irrespective of whether the pregnancy was wanted ($\chi^2 = 0.3$ at 1 d.f.).

The explanation of the high percentage of smokers among women with induced abortions can thus, at least partly be explained by the high proportion of unwanted pregnancies in this group. This phenomenon may also affect the number of smokers among women who abort spontaneously. Among 432 such women where it was stated whether the pregnancy was wanted, 117 (27%) reported an unwanted pregnancy a significantly higher proportion than among women who gave birth to live-born, normal children (15%)—$\chi^2 = 55.0$ at 1 d.f. $P < 0.001$

In Table IV the number of smokers is compared among women with wanted and unwanted pregnancies and with spontaneous abortion and with live-born, normal children. A χ^2 analysis of heterogeneity due to pregnancy result in the same acceptance of the pregnancy gives: χ^2 5.9 at 2 d.f., which corresponds to P value just above 0.05 and χ^2 analysis of heterogeneity due to desideration of the pregnancy with the same pregnancy result gives $\chi^2 = 35.9$ at d.f. $P < 0.001$. Practically all differences between smoking frequencies in women with spontaneous abortions and with normal, live-born children are thus due to differences in the mothers acceptance of the pregnancy and only a border-line significance remains when desideration is taken into consideration. This possible effect can perhaps be coupled to some other source of variation, as yet unidentified.

Table IV. *Effect of smoking and desideration on abortion*

Pregnancy result	Desideration	Number of women	Number smoking	Per cent smoking
Spontaneous abortion	Wanted	313	146	47
	Unwanted	117	71	61
Live-born, normal child	Wanted	4 134	1 719	41.6
	Unwanted	712	373	52

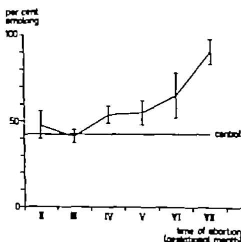


Fig. 1. Graph showing frequency ith errors, of omen smoking. Women ith spontaneous abortions, plotted against time of abortion, expressed as gestational month. Control line gives smoking frequency among women having non-malformed child, surviving to 1 year of age.

A study of the smoking habits of women with spontaneous abortions according to the gestational month when abortion occurred shows steady increase (Fig. 1). Thus, women aborting during the second or third gestational month show the same incidence of smoking as women who go on to have a normal child surviving to 1 year of age. Women who have later abortions, however show an increased incidence of smoking. Women who go on to have a legal abortion also show an increased incidence of smoking irrespective of when during the pregnancy the abortion occurs. This observation decidedly argues against an effect of smoking on early spontaneous abortions.

2. *Malformations and death rate*

The frequency of smoking among women having living and dead children—each group divided into non-malformed children, children with only minor malformations, and children with major malformations—is given in Table V. A χ^2 analysis for heterogeneity due to malformation group with the same survival is 1.7 at 3 d.f. $0.7 > P > 0.5$. There is thus no evidence for an effect of smoking on rate of minor or major malformations. The actual frequency of children with only minor malformations among children born to smoking mothers is 10.0% and that among children born

Table III *Smoking habits in women with an unwanted or a wanted pregnancy who gave birth to a live-born, normal child*

Desideration of pregnancy	Number of women	Number smoking	Women with unchanged smoking habits: Total number	< 10 cigs/day	> 10 cigs/day
Wanted	4 134	1 719	1 027	835	194
Unwanted	712	373	229	167	62

of the woman were classified in one of four groups no smoking, average less than 10 cigarettes per day average 10–19 per day and average 20 or more per day The amount smoked was given for each gestational month (4-week periods after LMP).

The population studied is racially uniform, practically all being Caucasian. 6 913 pregnancies were recorded, but only 6 376 (92.2 %) could be followed. *Table I* records the smoking habits of these women.

The outcome of the pregnancy was evaluated from the hospital records as spontaneous abortion (before the VIIIth gestational month) induced abortion (legal and known criminal abortions) extrauterine pregnancy birth of a child. Table II shows the actual numbers of different pregnancy types. The group of extrauterine pregnancies is too small to permit meaningful analysis and will not be discussed further

The presence of major or minor malformations (cf. Ekelund et al., 1970), birth weight, head and shoulder circumf rence, body length, and placenta weight were recorded. Children were followed up to the age of 1 year Stillbirths and deaths during the first year were all investigated by autopsy The age t death was recorded.

The smoking habits were compared with various other data. From the questionnaires was collected information about whether the women had had morning sickness furthermore—only on the first questionnaire—whether the pregnancy was wanted or not. The age f the woman at delivery and her social class were obtained from the birth record The social class was evaluated by the health insurance class f the woman or her husband, whoever had the highest. As health insurance is compulsory in S eden and the sum paid is proportional to the person s income, this gives an estimat of economic status.

Information on parity and signs of preeclamptic complications or toxaemia was obtained from hospital records.

All this data—and much more not used in the present study—was fed into a computer by IBM Stockholm.

RESULTS

1 *Abortion*

As Table II shows, _076 women, out of 4 903 smoked and had a completely normal pregnancy that is to say had a child without any identified malformations, either minor or major and alive at the age of 1 year This figure (42.3 %) compared with the frequency of smoking among women who have had a spontaneous abortion shows the latter rate to be significantly higher ($\chi^2 = 12.5$ at 1 d.f., $P < 0.001$) The same is true, however also for women who have an induced abortion ($\chi^2 = 11.7$ at 1 d.f., $P < 0.001$). The frequency of smokers is even higher in the latter group than among women with spontaneous abortions, but this difference is not statistically significant ($\chi^2 = 1.2$ at 1 d.f.)

No direct causal relation between smoking and induced abortion seems to exist. The latter group of women is especially characterized by their pregnancies, in most cases, being unwanted. It is highly probable that a woman with an unwanted pregnancy is more likely to smoke than a woman with a wanted pregnancy In order to study this possibility the possible association between smoking and whether the child was wanted was studied in a reference group of women who had live-born children without any malformations, either minor or major The reason for choosing women *with live-born children* and not women with children living at 1 year of age is that the post natal life of a live-born child may be influenced by social factors related to whether or not it was wanted. Table III reports the results of this study Information about both whether the pregnancy was wanted *and* smoking habits was available for a total of 4 843 such women Among those reporting wanted pregnancies, 41.5 were smokers among those reporting unwanted pregnancies, 52 4 . This difference is statistically highly significant (χ = ?9 1 at 1 d f P 0 001) *Among* the 1 256 women of this group who reported unchanged smoking habits during the pregnancy the degree of desideration was associated with amount smoked. Thus, among women with a wanted pregnancy 18 9 % of the continual smok

Table VII. *Effect of smoking on prematurity rate (as defined by birth weight (2 500 g). Live-born, normal infants born to women with unchanged smoking habits*

Smoking habits	No. of women	No. of premature births	Prematurity rate
No smoking	2 823	92	3.3%
10 cigs/day	1 031	50	4.8 %
10–20 cigs/day	261	12	5.6 %
> 20 cigs/day	9	3	

children dying before 1 week of age reveals the following: abruptio placentae was found in 12 of the 62 children born to smoking women but only in 2 of the 58 born to non-smoking women. A Fisher's exact test gives $P = 0.006$. Thus there are more placental abruptions among cases of perinatal death born to smoking mothers than to non-smoking mothers. In this connection, it can be noted that three of these mothers showed generalized fibrinolysis related to their abruption—two were moderate smokers and one a heavy smoker.

Concerning children dying after the age of 1 week, no specific cause of death can be found. In 4 of the 12 children whose mothers smoked, the cause of death was probably unrelated to smoking—3 were mongols, one had Mb. Oppenheim. Similarly 2 of the 6 children born to non-smoking mothers had mongolism. The rate of deaths from other causes is thus 8 of 2 396 children of smoking mothers and 4 of 3 224 children of non-smoking mothers. Among these, the following causes of death are found: congenital heart disease (4 against 3), respiratory tract infection (3 against 1), and generalized bleedings (1 against none). The material is too small to permit conclusions.

3 *Birth weight*

The effect of smoking on birth weight was studied on those pregnancies which resulted in the birth of a living child without any malformations, minor or major. Among a total of 4 947 such children, 193 had birth weight below 2 500 g. Such children will be called premature but this refers only to their birth weight. 2 124 women were smokers, and 101 of them had a premature child, of 2 823 non-smoking women, 92 had a premature child. This difference in frequency is statistically significant ($\chi^2 = 7.2$ at 1 d.f. $0.01 > P > 0.001$).

The quantitative effect of smoking on prematurity rate was analysed by comparing the non-smoking group with women who reported unchanged smoking habits during the pregnancy. Again, only those pregnancies that ended with the birth of normal, live-born child were included. Table VII lists the results. There is an increase in the numbers of low birth weight infants with increasing cigarette consumption. Women smoking less than 10 cigarettes a day also show an increased prematurity rate ($\chi^2 = 5.4$ at 1 d.f., $P = 0.02$).

The mean length of gestation was the same for smoking as for non-smoking women. The premature children are thus really 'small-for-dates'. The distribution of child death among premature children and non-premature children and among smoking and non-smoking women is shown in Table VIII. As is obvious from this Table, the slight but statistically not significant increase in stillbirth rate is the same for both premature and mature children. The statistically significant effect on post-partum death rates, however is observable only among non-premature children. This would favour the hypothesis that smoking has an effect on the survival of live-born child ir

Table VIII. *Effect of smoking on survival and prematurity*

		Mother smoking		Mother not smoking			
Time of death	Birth weight (g)	No. of children at risk	No. of children dead	No. of children at risk	No. of children dead	χ^2	Ratio
Stillborn	2 500	113	14	102	10	0.1	1.2
	2 500	2 343	18	3 180	22	0.1	1.1
After birth	2 500	101	16	92	13	0.0	1.0
	> 2 500	2 323	26	3 158	17	5.8	2.1

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Table V *Smoking habits related to survival and malformations in children*

	Total	Number smoking	Per cent smoking
Children surviving 1 year of age	5 603	[illegible] 384	4[illegible]5
of these. normal children	4 903	2 076	4[illegible]3
children with minor malformations only	550	245	44.5
children with major malformations	150	63	42.0
Children dead before 1 year of age	137	74	54
of these: normal children	94	48	52
children with minor malformations only	1	1	—
children with major malformations	42	25	60

to non-smoking mothers 9.3%. Corresponding frequencies for children with major malformations is 3 6 and 3 2% resp.

χ^2 for heterogeneity due to survival within the malformation group is 6 93 at 2 d.f., $0.05 > P > 0.02$. If the number of smokers is compared among all women with children surviving to 1 year of age and women with children dead before one year of age, $\chi^2 = 7.2$ at 1 d.f., $0.01 > P > 0.001$. There is thus a higher rate of smoking in women who have a baby that dies before the age of 1 year than in women who have a baby surviving at 1 year and this is not due to an effect on malformation rate

The time of death of the children of smoking and non-smoking women is shown in Table VI where a ratio is also shown which indicates the increase in death risk connected with smoking. The ratio is obtained by dividing the death rate in children born to smoking mothers with that in children born to non-smoking mothers. This Table indicates a continuous increase in death risk with increasing age of the baby but the actual figures are small and the significance uncertain. If all stillborn children are regarded as one group 32 have died out of 2 458 children of smoking women and 32 out of 3 282 children of non-smoking women. No statistical significance exists for this slight difference. If all perinatal deaths (before 1 week of age) are compared in the two groups, the ratios will instead be 62 2 458 and 58 3 282—these differences are of some statistical significance, $\chi^2 = 3.9$ at 1 d.f., $0.05 > P > 0.02$.

The death rate among children of smoking women is thus slightly higher and this increase is evident both in the period before the first week and that after the first week, but no definite evidence for an increased rate of stillbirths was found. This agrees with the results of Comstock & Lundin (1967). The present material indicates that the death risk increases with the age of the children, but this finding is statistically uncertain.

An examination of the cause of death of the

Table VI. *Smoking and death risks at different child age*

Time of death	Mother smoking: Number of children at risk	Mother smoking: Number of children dead	Mother not smoking: Number of children at risk	Mother not smoking: Number of children dead	χ^2	Ratio
Before birth	458	1	3 [illegible]82	[illegible]3	0 4	1.2
During birth	2 437	11	3 259	9	1.[illegible]	1 6
During first week	2 4[illegible]6	30	3 [illegible]50	[illegible]6	2 7	1.5
After first week	396	1	3 224	6	4.3	2.7

0.05 > *P* > 0.02.

Table XII. *Effect of various variables on prematurity rates*

A. *Complete subdivision of variance sources*

Between smoking within age, parity, and desideration: $\chi^2 = 14.9$ at 6 d.f., $0.05 > P > 0.02$

Between parity within age, desideration, and smoking: $\chi^2 = 15.8$ at 6 d.f., $0.02 > P > 0.01$

Between age within parity, desideration, and smoking: $\chi^2 = 6.0$ at 5 d.f., $0.5 > P > 0.3$

Between desideration within parity, age, and smoking: $\chi^2 = 5.5$ at 6 d.f., $0.5 > P > 0.3$

B. *Subdivision with respect only to parity and smoking*

Between smoking within parity: $\chi^2 = 14.8$ at 2 d.f., $P < 0.001$

Between parity within smoking: $\chi^2 = 9.8$ at 2 d.f., $0.01 > P > 0.001$

Interaction between smoking and parity: $\chi^2 = 0.8$ at 1 d.f., $0.5 > P > 0.3$

not affect prematurity. If, therefore, the effects of smoking within parity and of parity within smoking groups are studied (section B) both are shown to be significant, but no interaction exists between them.

The possible effect of social class has also been evaluated. Each subgroup was then split into five further subgroups, but these were so small that the information obtained is not very reliable. From the material available there are no indications that social groups affect prematurity rates except via parity and smoking.

A more sensitive test of the effect of the above-mentioned factors is to use the birth weights. The results are given in Table XIII. This Table shows that all factors independently affect birth weight (that is, irrespective of the other three factors). There is no indication of an interaction between smoking and the other sources of variation—

Table XIV. *Morning sickness and preeclampsia in women according to (unchanged) smoking habits. Live-born, normal children*

Smoking habits	Frequency of morning sickness	Frequency of preeclampsia
No smoking	2171/2813 = 77 %	440/2815 = 16 %
< 10 cigs/day	656/1018 = 64 %	109/1023 = 11 %
10–20 cigs/day	166/249 = 67 %	27/231 = 11 %
> 20 cigs/day	6/9	0/9
All smokers	828/1274 = 65 %	135/1282 = 11 %

the effect of smoking thus occurs in all subgroups in a similar manner.

4. Other effects of smoking on pregnancy

Table XIV shows the relation between smoking and frequency of morning sickness and preeclampsia. Both phenomena are less common among smokers than among non-smokers ($\chi^2 = 66.7$ and 19.0 resp., both at 1 d.f., both $P < 0.001$). There is no apparent correlation with amounts smoked for either of the two. The increased rate of dead children among smoking women cannot be explained by an increased frequency of toxaemic complications. Similarly, emesis or the use of antiemetic drugs cannot explain the disturbances of the pregnancies in smoking women.

The sex ratio (males/females) among live-born, normal children, born by non-smoking women, is 1.01; that among such children born to smoking women is 1.06. This difference is not statistically significant, but is at variance with Fraumari & Lundman (1964) findings. No reduction in mean Apgar score at one minute could be demonstrated between live-born normal children born

Table XIII. *Analysis of various factors on birth weight. All women who gave birth*

Source of variation	d.f.	Variance	F	P
Error within smoking, parity, age, and desideration	5 543	312 940	—	—
Between parity within age, desideration and smoking	8	271 529 285	868	< 0.001
Between age within parity, desideration and smoking	8	1 166 654	3.7	< 0.001
Between desideration within parity, age, and smoking	8	790 149	2.5	0.01–0.001
Between smoking within parity, age, and desideration	8	434 351	14.0	0.001
Interaction between smoking and other sources of variation	7	358 475	1.1	0.2

Table IX. *Birth weight and placental weight at different amounts smoked Live-born, normal children. Un-changed smoking habits Mean ± standard error of mean*

Smoking habits	Birth weight, g		Placental weight, g	Mean placental weight / Mean body weight (both sexes)
	Boys	Girls		
No smoking	3 568 ± 14	3 44 ± 14	598 ± 3	0.171
< 10 cigs/day	3 417 ± 23	3 [illegible]67 ± 23	584 ± 4	0.175
10–20 cigs/day	3 368 ± 46	3 256 ± 46	591 ± 7	0.178
> 20 cigs/day	2 970 ± 306	[illegible]775 ± [illegible]11	541 ± 30	0.188

Table X. *Body length and head and shoulder circumference at different amounts smoked Live-born, normal children Unchanged smoking habits. Mean ± standard error of mean*

Smoking habits	Body length, cm		Head circumference cm		Shoulder circumference cm	
	Boys	Girls	Boys	Girls	Boys	Girls
No smoking	51 1 ± 0.1	50.3 ± 0.1	34.9 ± 0.04	34.3 ± 0.04	37.6 ± 0.08	37.3 ± 0.07
< 10 cigs day	50.5 ± 0 1	49 6 ± 0.1	34 6 ± 0.07	34.0 ± 0.07	37 1 ± 0.1	36.5 ± 0.12
10–20 cigs day	50.4 ± 0.[illegible]	49 7 ± 0.[illegible]	34.6 ± 0.13	34 1 ± 0.13	37.0 ± 0.[illegible]4	36.7 ± 0.[illegible]3
> 20 cigs day	48 7 ± 1.3	47.3 ± 1 0	34 0 ± 0.87	33.0 ± 0.61	35 0 ± 1 63	34 0 ± 1.07

respective of its effect on birth weight. Table IX shows the effect of smoking on mean birth weight and mean placental weight Both variables show a decrease with increasing amounts smoked, more evident for birth weight than for placental weight, present both in boys and girls. The mean birth weight for the children of smoking women, irrespective of amount smoked, is 3 405 g for boys and 3 260 g for girls t test against the means for children of non-smoking women show a high degree of significance ($t = 6.3$ and 5 8 resp $P < 0\ 001$) The Table also gives the ratio mean placental weight/mean body weight (both sexes) This ratio shows a steady increase with amounts smoked, which shows that placental weight reduction is less pronounced than body weight reduction. Table X gives means for body length, head circumference, and shoulder circumference—all dimensions show decrease with amount smoked.

Table XI *Some characteristics of pregnancies and smoking habits Women with unchanged smoking habits during pregnancy and giving birth to normal live-born children. Mean ± standard error of mean*

Smoking habits	Age of woman	Mean number of earlier pregnancies
No smoking	[illegible]6.9 ± 0.10	0.99 ± 0.02
< 10 cigs/day	[illegible]5.1 ± 0.16	1.01 ± 0.04
10–20 cigs/day	25.[illegible] ± 0.3[illegible]	1.32 ± 0.10
> 20 cigs/day	[illegible]5.9 ± 0.39	2.11 ± 0.39

The effect of smoking on birth weight may be complicated by differences in age, parity and maternal desideration of the pregnancy (wanted or unwanted) between smoking and non-smoking women. Table XI shows that smoking women are actually a trifle younger but have a slightly higher number of earlier pregnancies than non-smoking women. A division into two age groups (< 25 years of age > 25 years of age) and into two parity groups (no earlier child and one or more earlier children) was therefore performed. A χ^2 analysis of smoking frequencies showed that the difference between wanted and unwanted pregnancies is highly significant also within age and parity groups ($\chi^2 = 20.9$ at 4 d.f $P < 0.001$), and similarly that the difference between age groups within parity and acceptance groups is highly significant ($\chi^2 = 114$ at 4 d.f $P < 0\ 001$), but there is no statistically significant effect of parity within age and acceptance groups ($\chi^2 = 8.9$ at 4 d.f $P > 0\ 05$). Table XII shows the effect of these three factors and smoking on prematurity rates. After a complete subdivision of the sources of variation (section A) a significant effect remains for smoking and parity but age and desideration do

ore frequent among women with an unwanted regnancy and that spontaneous abortions and on-acceptance of the pregnancy are associated, test was performed to see whether differences a desideration could explain the different abor ion rates in smoking and non-smoking women. This was found to be so—the main effect of smoking disappeared when correction was made for desideration, and only a slight and statistically just possible effect of smoking on abortion rate remains. This might be random or due to non-identified source of variation. It can, of course also indicate a slight abortifacient effect of smoking. When spontaneous abortions were studied from the aspect of when the abortion occurred, it was found that women with an early abortion show the same incidence of smoking as women *who go on to have normal child and that the* association between smoking and abortion is due to late abortions.

One possible association between non-acceptance of the pregnancy and spontaneous abortion is that considerable number of these abortions re indeed induced. Pettersson (1968) in a study of abortion in Sweden suggested that perhaps some 30% of abortions among unmarried women admitted to hospital are induced.

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to smoking and non-smoking women—the two means are 9 8 and 9 7 resp

DISCUSSION

An analysis of the effect of smoking on pregnancy can be complicated by various interactions between smoking and other factors of importance for the pregnancy Such interactions have been exemplified in the present study and efforts have been made to clarify at least some of them to make it possible to study the direct effect of smoking on fetal survival and development. This study is a prospective one, which limits its size, but which may result in less biased data than a retrospective study

The effect of smoking on prematurity and birth weight has been amply demonstrated in earlier studies and was also verified in the present investigation. Such an effect is found also in moderate smokers (less than 10 cigs./day) as is apparent from Tables VII and IX. The risk of prematurity is increased by 50% if the woman smokes. This is perhaps partly due to other variables associated with smoking Among those studied (maternal age, parity and the desideration of the mother) only parity showed a significant effect, but smoking had a significant effect on prematurity rate also irrespective of parity (Table XII. B)

The mean birth weight is reduced in children of smoking women this effect is also seen in moderate smokers (Table IX) The overall reduction in birth weight associated with smoking is 170 g in this study—roughly the same in both sexes, and calculated on live-born, non malformed children. Again, this effect is highly significant when the other sources of variation studied are eliminated (Table XIII) but on this variable there is an isolated effect also of the mother's age, her parity and whether the pregnancy is wanted. There might be a correlation between desideration of the pregnancy and other characteristics, e.g., nutrition and drug consumption, which might explain this effect. This complicated interaction lies outside the scope of the present study

There might be other sources of variation which were not included in this study but they are hardly of a magnitude that could explain the recorded effect of smoking on birth weight and prematurity rates.

The birth weight reduction is paralleled by corresponding reductions in other infant measurements studied and also in placental weight. Placental weight, however is less reduced than birth weight—the ratio between the two increases with increasing amounts smoked (Table IX). There is some evidence from the literature, however that placental function may also be impaired in smoking women (Tanaka, 1967)

Neither in this study nor in any other published study could any effect on malformation rates be found. However the studies are too small to permit a firm conclusion on the non-teratogenicity of smoking. A teratogenic effect leading to a moderate increase in occurrence of a specific malformation cannot be demonstrated in such studies.

Earlier studies have given variable results on the possible effect of smoking on child survival. In the present study a definite increase in child mortality was found among children born to smoking mothers compared with those born to non-smoking mothers. Among the former 3 01% of all children born died before 1 year of age among the latter 1.92% Thus total death rate was increased 1 6 times. A more detailed analysis of the time of death gives no significant—although an indicated—increase in stillbirth rate, but a significant increase in deaths before one week of age and also in deaths taking place between the age of 1 week and 1 year These results agree in the main with those of Comstock & Lundin (1967) who also found an effect only on death rates among live-born children. We also found that the deaths before the age of one week are at least to some extent explained by a significant increase in abruptio placentae. No specific cause of death was found among children dying after the age of one week. The increase in death rate was seen only in non-premature children.

The association between smoking and spontaneous abortion proved interesting. Zabriskie (1963) described an increased abortion rate among smoking women compared with non-smoking women. The data in our study confirmed this observation (Table II) A similar—and even slightly higher—increase in smoking frequency however was also found among women who later have an induced abortion. The latter group is mainly characterized by having an unwanted pregnancy As it could be shown that smoking is

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RUPTURE OF THE NORMAL SPLEEN OCCURRING AS A BIRTH INJURY

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Abstract. A case of rupture of the normal spleen as birth injury is reported. The child was born 2 eeks past term. There as vertex presentation, and no history of trauma could be elicited except that associated with normal spontaneous birth. Eight hours after birth the infant developed signs of shock, and it died 7 hours later. Autopsy showed haemoperitoneum from sub-capsular tear of the spleen at the insertion of the lieno-renal ligament. An explanation of the probable mechanism of this injury is given.

Rupture of the parenchymatous abdominal organs occurring as a birth injury is relatively rare but important cause of neonatal death. In a study of 000 fetal and neonatal deaths Potter (1940) found 28 lacerations of abdominal organs and in 4 of these the organ involved was the liver (1.2%) N case of splenic rupture was found. Brown, Dunbar & MacEwan (1962) found massive adrenal haemorrhage in 4 out of 709 autopsies on newborns (0.58%). Rupture of the kidneys is very rare (Ravich & Schell, 1961 Giedon, 1963).

Splenic rupture is a rare complication of birth trauma. Most often it has been associated with cases of splenomegaly from such causes as erythroblastosis fetalis (Sillanpää & Hakulinen, 1968), and, more rarely congenital leukaemia (Rhaney 1938) and congenital syphilis (Lundqvist, 1930).

Only 18 cases of rupture of the normal spleen have been reported (Sieber & Girdany 1958 Hottinger & Gilardi, 1958 Šrámek & Vedrová Kardova, 1959 Myers, 196., Giedon, 1963 Portillo, García-Recca, Mañé-Garzón & Lattaro, 1963 Burnell & Markey 1965 O'Neill, O'Brian & Hyun, 1965 Donellöf, 1966 Schwartz & Cohn, 1966 Bouyala & Aubrespy 1966, Eraklis, 1967 Delta, Eberstein & Rothenberg, 1968).

The following case, which ended fatally is published because it demonstrates a probable mechanism f this birth injury

CASE HISTORY

The mother was 31 year old oman ho had been treated ith primidone and phenytoin since 1964 because of epilepsy She had never received blood transfusion. She was primigravida. Her last menstrual period began on June 22, 1969 During pregnancy she was under the care of gynaecologist. Her blood group was O, Rh negative, and no immune antibodies could be detected. She was admitted to the maternity hospital on April 15, 1970 with regular labour pains. On admission the fetal heart sounds were normal. Nine hours later normal boy was born There had been a left occipito-anterior position of the vertex. No pressure had been applied to the maternal abdomen during labour. The birth eight was 3 960 g and baby length was 53 cm. The mother lost 1 500 ml of blood and the placenta had to be removed manually The placental weight as 550 g.

Eight hours after birth the child was found pale and hypotonic in its cot, and was therefore immediately transferred to the paediatric hospital. On admission it as pale with impaired peripheral circulation. The femoral pulse as soft with frequency of 80 per minute The abdomen was soft and there were no palpable tumours. The liver and spleen ere not palpable. Shortly afterwards respiration stopped, and he was intubated and ventilated. A catheter as introduced into the umbilical vein and 12 ml of bicarbonate and 17 ml of glucose ere given, hereupon the condition somewhat improved. The haemoglobin level in the blood from the umbilical vein was 74%. The infant blood group was the same as that of the mother O, Rh negative, and the direct Coombs test as negative. The child now received 40 ml of O, Rh negative blood, but without improvement in the general condition hich deteriorated rapidly He died 7 hours after admission to the paediatric hospital. Because of the normal abdominal findings on admission the correct clinical diagnosis as not made and laparotomy as not undertaken.

Autopsy was performed 12 hours after death. The abdomen contained 200 ml of fluid and coagulated blood. The abdominal organs ere first examined *in situ*. At

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CASE HISTORY

The mother was 31 year old woman who had been treated with primidone and phenytoin since 1964 because of epilepsy She had never received blood transfusion. She was primigravida. Her last menstrual period began on June 22, 1969 During pregnancy she was under the care of gynaecologist. Her blood group was O Rh negative, and no immune antibodies could be detected. She was admitted to the maternity hospital on April 15 1970 with regular labour pains. On admission the fetal heart sounds were normal. Nine hours later normal boy was born. There had been left occipito-anterior position of the vertex. No pressure had been applied to the maternal abdomen during labour. The birth weight was 3 960 g and baby's length was 53 cm. The mother lost 1 500 ml of blood and the placenta had to be removed manually The placental eight was 590 g.

Eight hours after birth the child was found pale and hypotonic in its cot, and was therefore immediately transferred to the paediatric hospital. On admission it was pale with impaired peripheral circulation. The femoral pulse was soft with frequency of 80 per minute The abdomen was soft and there were no palpable tumours The liver and spleen were not palpable. Shortly afterwards respiration stopped, and he was intubated and ventilated. A catheter was introduced into the umbilical vein and 12 ml of bicarbonate and 17 ml of glucose were given, whereupon the condition somewhat improved. The haemoglobin level in the blood from the umbilical vein was 74 %. The *infant* blood group was the same as that of the mother O, Rh negative, and the direct Coomb test as negative. The child now received 40 ml of O, Rh negative blood, but without improvement in the general condition hich deteriorated rapidly He died 7 hours after admission to the paediatric hospital. Because of the normal abdominal findings on admission the correct clinical diagnosis was not made and laparotomy was not undertaken.

Autopsy as performed 12 hours after death. The abdomen contained 200 ml of fluid and coagulated blood. The abdominal organs were first examined *in situ*. At



Fig 1 The lower pole of the spleen has been pulled forward and medially to expose the lieno-renal ligament. Note the short, thickened ligament infiltrated with blood and with a small oval hole in its lower surface. To the right is seen some blood clot that was slightly adherent to the lower pole of th pleen and the lower surface of the lieno-renal ligament.

the lower pole of the spleen coagulum was found. The spleen was less mobile than usual and could not be pulled forward into the abdominal wound because of a short lieno-renal ligament extending from the postero-lateral surface of the spleen onto the lower part of the diaphragm near its insertion into the lower ribs. This ligament was the seat of haematoma that had ruptured into the abdominal cavity through a small hole in its lower surface (Fig. 1). The ligament was carefully dissected free from the spleen, and on the posterior surface near the hilum was found small laceration the splenic surface probably caused by avulsion of the ligament from the spleen at this location.

The spleen weighed 15 g and was histologically normal. The macroscopic and microscopic xamination of the other organs failed to show any abnormality

DISCUSSION

In a study of 8 cases of rupture of the liver and spleen in the newborn Gruenwald (1948) found three different types of lacerations according to their position in the organs. One type was associated with peritoneal ligaments attached to tl liver and spleen, suggesting that an abnormal pu on these ligaments was a cause of the ruptur Another type was found on the anterior surfa of the liver indicating pressure from the cost margin, and finally instances were found in whic no specific explanation for the site of the damag could be given.

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Mountain, Hirsh & Gallus (1970) reported a increased bleeding tendency in infants born to mothers treated with certain anticonvulsant drugs. A coagulation defect similar to that in vitamin-K deficiency was found in 8 out of 16 neonates examined, and was severe in 7 Two of the neonates showed clinical signs of a bleeding tendency The mothers of the 8 affected neonates had been treated with a barbiturate, or with a drug metabolised to a barbiturate, and with phenytoin. The possibility of an increased bleeding tendency occurring in neonates born to mothers who are being treated with these anticonvulsants is therefore significant. The coagulation factors were not examined in this case, but it seems unlikely that a coagulation defect should be the sole cause of the splenic haemorrhage, since there were no clinical signs of a generalized bleeding tendency It is more likely that a coagulation defect might have augmented a haemorrhage brought on by avulsion of the lieno-renal ligament from the surface of the spleen.

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STUDIES ON FIBRINOLYTIC INHIBITORS DURING PREGNANCY

Ulla Hedner and Birger Åstedt

From the Coagulation Laboratory (Head: Professor Inga Marie Nilsson) and the Department of Obstetrics and Gynecology (Head: Professor Sten Kullander), Malmö General Hospital, Malmö, Sweden

Abstract. Each ideal fibrinolytic inhibitors (α_2-macroglobulin, antiplasmin, plasminogen activation inhibitors), their relative values and the variation of such values as well as that of plasminogen were measured in 120 women during normal pregnancy (40 in each trimester).

α_2-macroglobulin and antiplasmin were increased throughout pregnancy. The inhibitors of plasminogen activation were determined by means of both a clot method and caseinolytic method. The level remained largely unchanged during pregnancy. These findings were confirmed by the results obtained after gel filtration on Sephadex G-200 of sera from 25 pregnant women. The level of plasminogen was found to be high during the whole of pregnancy. Our findings suggest that the reduced fibrinolytic activity during pregnancy is due to a decreased production and/or defective release of plasminogen activators from the vessel walls.

The spontaneous fibrinolytic activity in the blood is substantially reduced during pregnancy (2, 3, 4, 13, 20, 27). The mechanism of this relatively low fibrinolytic activity in pregnancy is, however, still obscure, some workers ascribing it to an increased content of inhibitors in the blood (2, 13, 14, 18, 19, 22) and others, to a decreased production or release of plasminogen activators from the vessel walls (3, 4, 20, 28). This lack of unanimity is accentuated by the fact that some authors did not find any increase in inhibitors (1, 5, 23). In many of the investigations it is, however, not clear whether determinations were made of antiplasmin, antiactivator, antistreptokinase or inhibitors of plasminogen activation.

This paper concerns the individual inhibitors of fibrinolysis and the variation in their pattern during pregnancy. The investigation was extended to include determination of the plasminogen level.

CLINICAL MATERIAL AND BLOOD SAMPLING

The clinical material consisted of 120 apparently healthy pregnant women, 40 in each trimester. The samples were obtained at routine tests taken at the antenatal clinic. Sera from 40 healthy women, matched for age and not taking oral contraceptives, served as controls.

The blood was collected via the siliconе technique and serum was prepared in the way described previously (21).

METHODS

1. *α_2-macroglobulin* (α_2M) was measured with an esterolytic method (10). The result is expressed relative to the content of a normal standard consisting of pooled serum from 20 apparently healthy persons. Normal range: 80–150%.

2. *Antiplasmin* activity was assayed with Shamash & Rimon's (26) caseinolytic method, as modified by Ekelund et al. (7). Normal range: 400–600 ACU/ml.

3. *Inhibitors of urokinase activation of plasminogen* (urokinase inhibitors) were measured with (*a*) a clot method described by Paraskevas et al. (21). The result is expressed relative to the content of a normal standard consisting of pooled serum from 25 apparently healthy persons. Normal range 60–140%, and (*b*) a caseinolytic method described by Lassen (17), as somewhat modified by Hedner, Nilsson & Jacobsen (16). In this method the effect of antiplasmin is excluded. The result is given in extinction units and the normal range is 0.022–0.055.

4. *Total antitryptic activity (TAT)* was measured by Eriksson's (8) esterolytic method. Normal range 0.7–1.4 mg/ml.

5. *Plasminogen* was determined with Ganrot & Nilehn's (12) immunological method, as modified by Ekelund et al. (7). The blood is collected in tubes containing ε-aminocaproic acid (EACA) to avoid in vitro fibrinolysis. Normal range: 70–150%.

6. *Gel filtration* of sera from 25 pregnant women in the 3rd trimester on Sephadex G-200 (AB Pharmacia,

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Table I *Mean values of the different fibrinolytic inhibitors and of plasminogen in 120 pregnant and 40 non-pregnant women*

Inhibitor	Non-pregnant women	Pregnant women First trimester	Second trimester	Third trimester
α_2 macroglobulin,	110	137	132	121
Antiplasmin, ACU/ml	417	570*	601	671
A titrypsin,	114	127*	1 6	131
Inhibitors of plasminogen activation				
(a) Clot method,	111	118	103	79
(b) Caseinolytic method, ext	0.040	—	—	0.045
Plasminogen,	90	123	139	140*

) $p < 0.001$
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Uppsala) was performed at pH 7.8 with a 0.15 M Tris buffer For further details, see Hedner et al. (16). The results are compared with the normal pattern of the various fibrinolytic inhibitors after gel filtration (for details see Hedner et al. (16)).

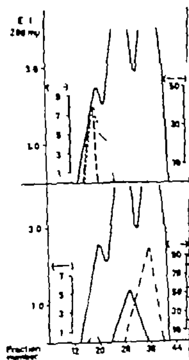


Fig 1 Mean values of fractionation of 5 ml serum from 4 healthy individuals. Sephadex G-200 0.15 M Tris buffer pH 7.8 inhibitor of plasminogen activation (%) --- α_2-macroglobulin (•) — — — antiplasmin (ACU/ml fraction) —— plasminogen (ACU/ml fraction).

RESULTS

1 *α_2-macroglobulin* (α_2M) was increased above non-pregnant values in each trimester The increase was largest during the first two trimesters ($p < 0.001$). A slight decrease was noted in the 3rd trimester

2. The *antiplasmin activity* was significantly increased throughout pregnancy ($p < 0.001$) It also rose successively and the average level during the 3rd trimester was significantly higher ($p < 0.001$), than that during the first.

3 *The inhibitors of urokinase activation of plasminogen* (urokinase inhibitors) did not differ significantly from normal during the first two trimesters. During the 3rd trimester however the *clot method*" showed a small but significant ($p < 0.001$) decrease to a mean value of 79 which is not below the lower limit of the normal range while corresponding values obtained with Lauritsen's *caseinolytic method* were normal also in the 3rd trimester (mean 0.045).

4 *Total antitrypsin activity (TAT)* was increased throughout pregnancy The higher values were noted during the 3rd trimester (p 0.001). No significant difference was found between the three trimesters in this respect.

5 *Plasminogen* was significantly in reased throughout pregnancy ($p < 0.001$) No significant difference was found between the trimesters

6. *Gel filtration* on Sephadex G 200 After gel filtration of normal sera (Fig. 1) the α M is seen as a well defined peak in the first protein zone

ontaining the proteins of high molecular weight. The urokinase inhibitors determined with the clot method gave two peaks: the first wide and plump and partly overlapping the α_2M-peak, the second, in front of and in the anterior part of, the albumin peak. The antiplasmin was accumulated in two peaks, a small one in the first protein zone and a larger one in the albumin peak. The separation pattern in the pregnant women (Fig. 2) showed no abnormality of the α_2M. As for the antiplasmin, the first peak was of normal configuration, while the second one was abnormally wide and high. The urokinase inhibitors showed normal first peak, while the second one was usually missing. In some of the cases there was a very small second peak. The total amount of urokinase inhibitors obtained in the first and the second peak, respectively, as determined with the clot method, was determined and compared with the corresponding values obtained after gel filtration of normal sera. No significant differences were found ($p > 0.05$). When examined with Lauritsen's caseinolytic method, the pattern of the urokinase inhibitors was normal (Fig. 3) as for the total amount of inhibitory activity in the two peaks ($p > 0.05$).

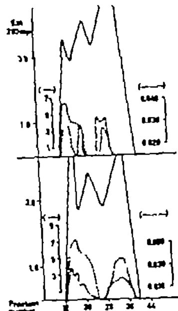


Fig. 3. Mean values of fractionation of 5 ml serum from 11 healthy individuals (above) and 25 pregnant women (below). ——, Inhibitor of plasminogen activation, clot method (%) — — caseinolytic method (ext.).

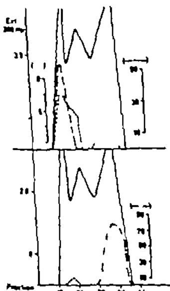


Fig. Mean values of fractionation of 5 ml serum from 25 pregnant women (3rd trimester). α_2-macroglobulin (%), inhibitor of plasminogen activation (%); — antiplasmin (ACU ml fraction).

DISCUSSION

As expected, the values found for α_2M, antiplasmin and total antitrypsin were high throughout pregnancy (9, 11, 14, 18, 20, 22, 24, 25). The relative fall in the concentration of α_2M during the last trimester found by us might be explained by advancing hydraemia towards the end of pregnancy. Some authors, however, have found no change in antiplasmin activity during pregnancy (1, 5). This difference might be explained by the fact that determinations were not made of the separate inhibitors and that the results may have been affected by the content of antiplasmin, antiactivators and inhibitors of plasminogen activation.

Opinions differ most regarding inhibitors of plasminogen activation during pregnancy. Thus, Brakman & Astrup (5) and Lauritsen (18) reported an increase of the inhibitors of urokinase activation of plasminogen, a finding that could not be corroborated by Nilsson & Kullander (20), Correll & Sjoerdsma (6) or Bonnar et al. (3). In the present investigation we determined the inhibitors of activation partly with a clot method

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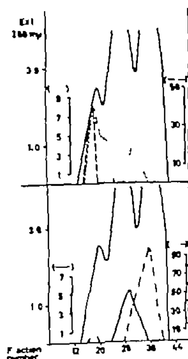


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in which a high concentration of antiplasmin and a high concentration of plasminogen may influence the values (21) and partly with a caseinolytic method in which the effect of antiplasmin (17) is eliminated With the clot method we found a very small but significant reduction of the urokinase inhibitors during the 3rd trimester But in practically all of the patients the values still lay within the normal range (mean 79%) In accordance herewith after gel filtration on Sephadex G 200 the inhibitors of activation produced at most a very small 2nd peak and an otherwise normal separation pattern. The slight reduction of urokinase inhibitors found during the 3rd trimester of pregnancy may be due to increasing hydraemia and/or elevation of the plasminogen Neither did the total inhibitory capacity in the first or second peak obtained on gel filtration differ from those found with normal sera. Tests with the caseinolytic method gave normal values also during the 3rd trimester (mean 0 047) The inhibitors of plasminogen activation were until recently a poorly defined type of inhibitors. Using different chromatographic methods we succeeded in isolating an α_2-fraction with a strong inhibitory effect on the activation of plasminogen. This fraction has also been found to be missing in some individuals with a high fibrinolytic capacity (16)

Our findings thus suggest that the activating inhibitors remain unchanged during pregnancy while α_2M and antiplasmin are substantially increased A high α_2M content is normally seen in infants in whom it is much higher than that seen during pregnancy Nevertheless, they have normal fibrinolytic activity (7) The antiplasmin level is often raised in reactive processes. But only exceptionally is the fibrinolytic activity reduced in such patients. Our findings thus suggest that the reduced fibrinolytic activity during pregnancy is due mainly to reduced production (3 4) or release of plasminogen activators from the vessel walls (28) and only to a minor extent to the inhibitors.

In our opinion the findings also suggest that the various inhibitors of fibrinolysis, α_2M antiplasmin and urokinase inhibitors, are of a different nature and show different reaction patterns.

Opinions differ also about the plasminogen level during pregnancy Thus Shaper et al. (27) and Brakman (4) found no difference between the plasminogen levels in pregnant and non-pregnant women, while Naidoo et al. (19) found them to be low during pregnancy Most authors have, however reported high values of plasminogen during pregnancy (15 18 20, 22) In this investigation we used an immunochemical method of determination and found a successive increase of the plasminogen during pregnancy i.e. a finding which is in agreement with the results of these latter authors.

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FORTY FIVE OPERATIONS FOR STERILITY

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Abstract The literature concerning operations for sterility is reviewed and personal series of 45 women, subjected to surgical treatment for sterility is presented. Traditional techniques have been employed, without the use of prophylactic antibiotics or steroids. The relevant data of all the omen is summarized. In the 45 patients treated, there were 23 pregnancies, 21 intrauterine and 2 extrauterine. Seventeen women had the 23 intrauterine pregnancies. I the 25 patient here salpingostomy alone as performed, there were 13 pregnancies, 11 intrauterine and 2 extrauterine or calculated by the number of women ho concerned, there ere 36% ith ultimate intrauterine pregnancies. The results are found to be satisfactory and surprising. The material is too small to make an assessment concerning the factors hich influence the prognosis, but the prognosis appears to be best in young women ho have not had previous surgery in the lower abdomen and ho have few adhesions. The duration of sterility or occurrence of previous pregnancies does not seem to have any prognostic significance. It is concluded that plast operations continue to have their place in the treatment of sterility and that the prognosis is somewhat better than usually considered.

The results of surgical treatment of sterility due to tubal occlusion have not shown any improvement during recent years. This has been demonstrated in the collected statistics of Swolin (1967) concerning the results of 43 investigators. The best results so far reported are those of Martius (1951), but according to Swolin (1967) these re questionable due to the fact, that they referred to cases here only division of adhesions of the salpinges was undertaken. Carter (1953) could not even produce a 10 success after surgery and during the first decade of his investigation, he found that the pregnancy rate was lower in a surgically treated series than in a non-treated series.

Different authors (Siegler & Hellmann, 1963 Siegler 1964 Swolin, 1967) point out, that published results are not comparable especially because the particular workers have not given sufficient detail of pre-operative investigations, of the selection of patients, of the nature of the operation, or of postoperative investigations. It is not always clear what is meant by a positive result, tubal patency or pregnancy From the technical point of view one can, of course, call an open tube a positive result, but for an infertile patient the only positive result is a living child.

Different attempts have been made to improve the above results by special techniques, e.g. atraumatic micro-technique in an operation field as bloodfree as possible (Swolin, 1967), application of intubation in one form or another (Green-Armytage, 1959 Shirodkar 1961 Vara, 1965). Some have used steroids systemically as well as locally Green-Armytage (1959), Sager (1964), Vara (1965), and Swolin (1967) have by this method demonstrated a significant reduction in the incidence of peritoneal adhesions, which has, undoubtedly improved the operative results in the treatment of closed tubes. Also antibiotics have been employed prophylactically in sterility operations (Green-Armytage, 1959 Henson et al., 1964 Swolin, 1967).

There seems to be greement (Stougaard Andresen, 1962 Swolin, 1967 Vara, 1965) that the best results are achieved when it is only necessary to divide tubal adhesions, poorer results occur following resection and reimplantation and the worst results are after salpingostomy

No special or new technique has been used in the present series, and the purpose of publishing the results is to show that the use of traditional techniques in medium sized special department, enables the achievement of results, which are not only acceptable, but which also are equal to the best results reported in the literature.

...her ...erances	Earlier operation in lower abdomen	Operation	Month and year	Patency postop +/+	Pregnancies: Miscarriages	Pregnancies: Live births	Pregnancies: Ectopic pregnancy	Remarks
	2	Suspensio uteri Salpingolysis bilat. adhes.	8 62	–				
	0	Salpingostomia bilat Resect. et implantatio adhes kath.	9 62	–		11/68		Microscopic diagnosis Salpingitis isthmica nodosa
	0	Salpingolysis Salpinx l normal adhes.	1 63					10/64 persufflation. (+) passage
?30 ...rtes	0	Salpinges norm lysis adhes appendectomia adhes.	2 63	–				
	0	Salpingostomia bilat + app Sectiosalp. bilat adhes.	2 63	–				
	1	Salpingostomia bilat lysis adhes adhes.	3 63	–		3/68		2/65 persufflation + passage new husband
?60 ...rtes	1	Enucleatio fibromatis, ventrosuspensio uteri, Salpinges norm 0 adhes	3 63					Adoption 6/66
	0	Salpingolysis l Salpingostomia l salpinx norm adhes	4 63					
	0	Salpingolysis et salpingostomia bilat appendectomia adhes	4 63					
	0	Salpingectomia l salpingostomia app adhes	5 63	–	4/68 b m III			2/64 persufflation. − passage
	0	Salpingostomia bilat adhes	6 63					Adoption 6/65
4 50, and 52 ...rtes	0	Salpingolysis bilat app Salpinges norm adhes	6 63					9/65 Salpingography Sactosalpinx Occl l
	0	Salpingostomia Salpingolysis l app adhes	8 63					
... 50 partus ...	0	Salpingostomia l Resect et implantatio tubae 0 adhes kath	8 63					11/65 Salpingography Occl passage l bot peritoneal changes
3	0	Salpingostomia bilat 0 adhes	8 63			pregnant		
32 ab	0	Salpingolysis bilat. app adhes	9 63			11/64 7/66		
58 ...	0	Salpingostomia bilat app adhes	10 63		12/63 ab m II			

Table I

Patient no	Age	Duration of sterility months	Salpingography No	Salpingography Result	Clinical investigation	Basal body temp	Premenstr endometrial biopsy	Sperma count and quality	Remarks
1	29	96	1	Normal	*Retroflex. uteri*	*Difas.*	*Reduced secretion*	*Normal*	*1949 resectio o. punctio ovarii et app. 1952 punct. ovarii l.*
2	24	42	1	Occl. salp. r Sactosalp. l.	Normal	—	—	Normal	
3	25	60	2	Occlusio r Adhesions	Cervicitis	Difas.	Normal	Normal	
4	30	96	2	*Occl. bilat.*	*Cervicitis*	*Monofas.*	—	*Normal*	
5	30	132	2	Occl. bilat. Sactosalp. l.	Cystic intumesc. behind uterus	Difas.	Normal	25 mill./ml 45 % immobil. 30 abnormal	1949, 1950, and 19[illegible] Salpingitis
6	25	46	1	Occl. bilat.	Normal	Difas.	—	Normal	1960 Salping. appendectomia
7	35	24	–	—	Retroflexion	Monofas.	Normal	—	1958 Webster+app. Salpinges normal Concipated after operat.
8	36	108	1	Sactosalp. l. Occl. r	Normal	—	—	Normal	
9	28	114	1	Sactosalp. l. Organic changes r	Normal	—	—	Normal	
10	25	72	1	Occl. l. Passage r	Hypoplasia uteri	Difas.	—	Normal	1 month preoperatively salpingitis l.
11	24	54	2	Sactosalp. l. Occl.	Normal	Difas	—	Normal	
12	32	132	2	Occl. bilat.	Cervicitis	Difas.	—	—	1st marriage 2 child. 2nd marriage 1 child but dead after 1 [illegible]
13	27	60	1	Sactosalp. l. Normal salp.	Normal	Difas.	Transitionfase	30 mill./ml 10 „ immobil. 40 abnormal	
14	33	96	1	Sactosalp. l. Isthmusstenosis r	Normal	Difas	Normal	17 mill./ml 25 immobil. 20 abnormal	Tuberculous pulm. operata 1956
15	30	54	1	Sactosalp. l. Occl.	Normal	—	Normal	37 mill./ml 40 immobil. 20 abnormal	1955 and 1961 Salpingitis
16	31	48	1	Sactosalp. l. Organic changes	Normal	Difas	Proliferatio	19 mill./ml 15 % immobil. 50 „ abnormal	
17	24	28	1	Occl. bilat.	Normal	—	—	48 mill./ml 20 % immobil. 30 % abnormal	1961 Salpingitis gonorrhoea

rlier pregnancies	Earlier operation in lower abdomen	Operation	Month and year	Patency postop. +/+	Pregnancies: Miscarriages	Pregnancies: Live births	Pregnancies: Ectopic pregnancy	Remarks
	0	Salpingolysis + salpingostomia Resectio et implantatio L + kath. + adhes.	11 63	–		5/67		8/65 HSG Occl. tub. bilat.
	0	Salpingostomia bilat. Myomectomia adhes.	3 64	–				
	0	Salpingostomia + app L salpinx normal 0 adhes.	3 64	–				
) partus 1 grav. extraut.	0	Salpingostomia bilat. + app. + adhes.	5 64	–		9/67		
) partus	0	Salpingectomia L Salpingostomia + + adhes.	5 64	–				Pyosalpinx L 10/65 HSG sactosalp r.
	0	Salpingostomia bilat. adhes.	5 64	–				5? child adopted 8/68 Laparoscopi tube normal, L tube covered by adhesions
	0	Salpingostomia bilat. Lysis adhes adhes.	6 64	+				
	0	Salpingostomia bilat 0 adhes	10 64	+				2/66 Persuffl. + pass.
	0	Expl lap app Tubes normal 0 adhes.	10 64	–	10/68 m III			4/65 Persuffl. + pass. 5–6–7/68 Physex before conception
0 partus	0	Salpingostomia Salpingolysis l adhes.	1 65			7/66 9/68		
4 partus 4 gra. extraut.	1	Susp. uteri, Resectio ovarii pp salpinx normal adhes.	1 65	–				
52 b	0	Salpingostomia bilat 0 adhes	2 65		3/66 m III	5/68	6/65 10/65	See text.
59 b	0	Salpingostomia bilat. adhes	3 65					12/67 Persuffl. + pass.
)	0	Salpingostomia bilat. adhes	6 65					
55 partus 56 ab 58 partus	0	Salpingostomia L, salpinx het. Graviditas ovarialis. salpinx covered by adhes adhes.	6 65		9 66			
53 partus 61 partus	0	Salpingostomia l salpinx het. Suspensio uteri, app adhes.	8 65			12/66		
59 partus	1	Salpingostomia adhes.	10 65					7/66 Persuffl. pass.

Table I (*continued*)

Patient no.	Age	Duration of sterility months	Salpingography No.	Salpingography Result	Clinical investigation	Basal body temp	Premenstr endometrial biopsy	Sperma count and quality	Remarks
18	23	36	3	Sactosalp. r ? Occl. l.	Normal	Difas.	Normal	Normal	
19	26	36	2	Occl. bilat.	Normal	Difas.	—	14 mill./ml 20 % immobil. 43 % abnormal	
20	34	42	1	Sactosalp. r ? Organic changes	Normal	—	—	18 mill./ml 20 % immobil. 25 % abnormal	
21	29	84	3	Sactosalp. r Occl. l.	Normal	—	—	Normal	1950 partus: child adopted
22	23	20	2	Occl. bilat.	Normal	—	—	—	Persorfil. preoperative — passage
23	30	90	2	Occl. bilat.? Organic changes	Normal	Difas.	—	Normal	1955 endometritis
24	32	48	1	Sactosalp. bilat.	Kolpitis Trichomonas	—	—	22 mill./ml 10 % immobil. 15 % abnormal	1951 Gonorrhoea 1951 + 1953 Salpingitis
25	21	12	2	Sactosalp. bilat.	Cervicit chr	Difas.	Normal	Normal	1961 salpingitis
26	28	36	1 2	Occl. l. Normal r Organic changes	Normal	Difas.	Normal	Normal	
27	34	120	1 2	Sactosalp. l. Sactosalp. r	Normal	Difas.	—	Normal	1950 child adopted 1952 Gonorrh. + salpingit 1953 salpingitis r 8/64 inflamm. acute after salpingography
28	28	12	1	Normal r tube — l. tube	Retroflexio	—	Normal	Normal	1958 Salpingectomi 1961 Gonorrhoea 1962 Cervicitis
29	23	18	.	Sactosalp. bilat.	Normal	Difas.	—	—	1962 + 1963 Cervicit.
30	23	24	2	Sactosalp. bilat.	Normal	Difas.	—	Normal	Chronic low abdominal pains
31	26	12	1	Sactosalp. bilat.	Cervicitis Parametritis	Difas.	—	1 mill./ml 40 immobil. 50 abnormal	1962 Salpingitis lat. Salpingo-oophoritis
32	29	28	1	Organic changes	Intumescens behind uterus	Monofas.	Normal	—	1963 Cervicitis chr
33	26	24	1	Normal?	Retroflexio uteri	Monofas.	Proliferation	—	4/64 Physex Normal endometrial biopsy 3/65 Chlorampheni
34	26	36	2	Occlusio r	Normal	—	—	—	1960 Salpingectomia l. 1961 Salpingo-oophoritis

[illegible] pregnancies	Earlier operation in lower abdomen	Operation	Month and year	Patency postop. +/+	Pregnancies: Miscarriages	Live births	Ectopic pregnancy	Remarks
	1	Salpingostomia bilat. Suspensio uteri + + adhes.	11 65	+				4/67 Pertubation + pass.
	0	Salpingectomia Salpingostomia l. 0 adhes.	11 65	–	9 66	pregnant		Tubarian graviditet at operation
1 partus	0	Myomectomia + Suspensio uteri Endometriosis in Fossa Dougl. Normal salpinges. + adhes.	1 66	–				
2 ab	0	Salpingolysis bilat. Suspensio uteri adhes.	1 66	–		10/66		
	0	Salpingolysis bilat. Suspensio uteri adhes. + endometriosis	1 66	–				4/67 HSG passage? Pertubation + passage
2 ab 3	0	Salpingostomia bilat. adhes.	3 66	+				
[illegible]	0	Resectio et implant. [illegible] 1 tube shrunk and [illegible] in medial / + [illegible] adhes + [illegible]	3 66	–		12/67		
[illegible]	0	Salpingostomia bilat. adhes.	4 66	+				
2 partus ?	0	Salpingostomia bilat. Resectio ovarii l. + adhes.	5 66	–				At the operation an ovarian pregnancy was found. 2/68 Pertubation + pass.
1 ab prev. [illegible]	1	Salpingostomia Resectio ovarii 0 adhes.	6 66					3/67 Pertubation + pass.
1 partus	0	Salpingostomia bilat. app. adhes.	9 66	–		11/68		

[illegible], a sperm examination was often neglected because the husband had previously paternity; in some cases the hysterosalpingography precluded the need for further investigation. Laparoscopy was not used during the time of the above study.

Indications for operation were signs of tubal occlusion, hydrosalpinx or other anatomical changes which are possible causes of sterility. The patient was always informed that the prognosis was doubtful, but no woman who desired an operation was refused. No patients were rejected because of especially bad prognosis. During surgery a plastic repair on the tubes was abandoned because the chance for a positive result was considered impossible as for example in cases of severe adhesions.

The procedures have been the usual methods without special technique. Salpingostomy, salpingolysis, resection and reimplantation in utero have been done or combination of some of these procedures. In 5 cases (patients 4, 7, 26, 28 and 37) the operation was initially an exploratory laparotomy. Among these patients myomectomy and ventrosuspension was performed on 2 occasions, division of adhesions and appendectomy once, ventrosuspension, ovarian resection and appendectomy once, and appendectomy only once. Of these cases only the latter conceived subsequent to the laparotomy. 32 of the operations were performed by one person, and the remaining 13 were undertaken by 4 different surgeons. The exact choice of procedure was made during the actual operation, so that the best method was selected individually.

The procedures included 25 bilateral salpingostomies or

Table I (*continued*)

Patient no	Age	Duration of Sterility Months	Salpingography No.	Salpingography Result	Clinical investigation	Basal body temp	Premenstr endo-metrial biopsy	Sperma count and quality	Remarks
35	—	42	2	Sactosalp. l. Passage r	Normal	Difas.	Normal	Normal	1958 Abscess periapp liter appendectomi
36	29	72	1	Sactosalp bilat.	Normal	Difas.	*Normal*	—	*1953 Gonorrhoea*
37	40	24	1 2	Occl.? Normal	Retroflexio Fibroma uteri	Difas.	*Normal*	—	
38	33	48	1	÷ passage	Normal	—	—	—	Persoufflation pre-operatively + pass
39	30	48	2	Occl. bilat	Endometrio-sis	Difas.	Normal	Normal	Persoufflation pre-operatively ÷ pass
40	31	36		*Sactosalp. bilat.*	*Normal*	*Difas.*	*Normal*	*Normal*	*1950–57–59 salpingitis*
41	25	18	2	Organic changes	Normal	Difas.	Normal	Normal	Persoufflat on pre-operatively + pass
42	34	60	1	Sactosalp. bilat.	Normal	—	—	Normal	1949–58 Salpingitis
43	?7	18	1	Passage	Cystis ovarii	Difas.	Normal	Normal	1955 Gonorrhoea
44	7	36	1	Sactosalp. r — l. salp	Cystis ovarii	—	—	Normal	1965 Salpingo-oophorectomi l.
45	28	60	2	Sactosalp l. Organic changes	Normal	Difas.	Normal	Normal	1963 Metrorrhagia Cervicitis

MATERIAL AND METHODS

In 1966, when the department of obstetrics and gynaecology had been in existence for 4 years, it was felt that it would be interesting to evaluate the results in all the women, who had come to the department because of sterility. Such an assessment was made during 1966–1967 and the results will be published later. However the surgically treated patients are of special interest, and the present paper is limited to these.

The series comprises 45 women treated between 196_ 1966. Four patients were first operated on after the agreed period but have been included because they initially sought treatment in the department during that period. The ages of the women ranged from 1 to 40 years with an average of 8 years. The duration of sterility was from 1 to 13 months with an average of 53 months. 4 women had primary sterility and 1 secondary sterility. The latter group of secondary sterile patients had a total of 18 previous births, 10 previous abortions and previous ectopic pregnancies. Seven women had previously had pelvic or lower abdominal operations. 18 women had a history of gynaecological disease, such as salpingitis, endometritis and gonorrhoea which could have resulted in sterility.

The routine examination included general clinical and gynaecological examination, hystero-salpingography which was often repeated, basal temperature recorded over three menstrual cycles, premenstrual endometrial biopsy and examination of the husbands sperm. As is shown, it was not always necessary to perform all the investigations on all the patients. Reasons for this can be del

women who did not conceive was 28–73 months, with an average of 51 months.

Immediately after the operation and prior to discharge, insufflation was performed on 17 patients, all of whom had good passage of gas through the tubes. Insufflation was carried out later on 8 of the women who did not conceive (9–33 months postoperative, average 18 months) passage of gas was shown in 7 and doubtful passage in 1. Hysterography was performed on 4 women who did not conceive, one was found to have a right hydrosalpinx and left tubal occlusion 27 months postoperatively. Another patient, also investigated 27 months postoperatively was found to have a patent left tube but occlusion of the right tube. The third patient was found to have a hydrosalpinx in the only remaining tube 17 months postoperatively. The fourth patient was found to have doubtful passage of dye 15 months postoperatively but insufflation was positive. Finally there was one woman on whom laparoscopy was performed 51 months postoperatively. This revealed a normal right tube and the left tube embedded in adhesions. It must be concluded that tubal patency was demonstrated in 9 cases, and to these must be added the 17 women who demonstrated patency by conception. Thus a total of 26 women (58%) had tubal patency. However this may be a falsely low figure, as it must be remembered that 16 patients were not reexamined.

DISCUSSION

Considering the background of the results in the literature and our own pessimism in the department concerning operations for sterility our results seem to us to be extremely satisfactory and surprising. Our pessimism also had repercussions in the prognosis we gave to the patients prior to operation, namely that of 5–10% chance of a successful result. The patient herself made the decision whether or not to submit to operation, despite the poor prognosis, but all received the offer of surgical treatment, irrespective of the changes discovered during the investigations. It was considered reasonable to regard an intrauterine pregnancy even if it ended in abortion, as a positive result since there was evidence of an ovum having traversed the Fallopian tube. Conversely an extrauterine pregnancy must be considered as a negative result.

Table IV

Age group (y.)	No. of cases	Conceptions
21–25	13	7
26–30	20	7
31–35	10	3
36–40	2	0

Table V

Adhesions	No. of cases	Conceptions
+++	9	2
++	15	3
+	11	7
0	10	5

Table VI. *19 pregnancies in 19 women*

Intrauterine pregnancies		13
Children	11	
Abortions	2	
Extrauterine pregnancies		6

Our results may be compared with those of Swolin (1967). Forty-five women were treated surgically and 17 (38%) achieved intrauterine pregnancies, compared with 22% in Swolin's series of 50 cases. In the 25 patients subjected to salpingostomy 9 (36%) had subsequent intrauterine pregnancies, compared with 6 (18,2%) in Swolin's 33 patients. It is possible that the two series are not directly comparable, e.g. according to Swolin there are more serious cases in his series. 96% of Swolin's women had ++ or +++ adhesions, compared with only 53.3% in our series. Also he performed salpingostomy in 66% cases, against our 56%. On the other hand, it must be stated that in all the cases an operation on the tubes was undertaken even when changes were so severe that the prognosis was quite pessimistic. In addition, our series has the disadvantage that 10 of the husbands of the women in the investigation had hypospermia.

The results can be compared also with a second

Table II *23 pregnancies in 17 women*

Intrauterine pregnancies		21
Children	13	
Pregnant at the time of writing	2	
Abortions	6	
Extrauterine pregnancies		2

Table III *13 pregnancies in 9 women*

Intrauterine pregnancies		11
Children	5	
Pregnant at the time of writing	2	
Abortions	4	
Extrauterine pregnancies		2

salpingostomy on the only remaining salpinx, 4 unilateral salpingostomies where the opposite salpinx was normal, 2 salpingostomies on one side and salpingolysis on the other salpingolysis in 5 cases, salpingolysis on one side in one case where the opposite tube was normal, salpingostomy on one side and resection and reimplantation on the other side in 2 cases and unilateral resection and reimplantation in 1 case—as well as the previously mentioned laparotomies.

In 4 cases resection and reimplantation of the tube was followed by insertion of a plastic catheter

Neither steroids nor antibiotics were used prophylactically

Table I shows the relevant data concerning investigations, the history the nature of operation, the findings at laparotomy and the grading of the adhesions by Swolin s (1967) criteria. 0 = no adhesions, + = slight adhesions, + + = moderate adhesions, and + + + = excessive adhesions. Finally the results of pregnancies and postoperative investigations are shown.

RESULTS

The results are judged more by the number of subsequent pregnancies than by postoperative investigation which, as will be shown later was carried out in only a few cases.

In the whole series 23 pregnancies occurred, in 17 women as shown in Table II. Eleven of the 17 women who conceived had at least one successful pregnancy and two were still pregnant at the time of writing. Four patients conceptions ended in abortion.

Case 29 requires further explanation. Bilateral salpingostomy was performed on this patient in February 1965 In July she was admitted with a left-sided tubal pregnancy for which a salpingectomy was performed. In october 1965 she was again admitted with a diagnosis of right-sided tubal pregnancy based on the presence of a swelling and endometrial histology which showed pregnancy hyperplasia and Arias Stella phenomenon The swelling disappeared with conservative treatment. The HCG test was negative, showing that there was no active chorionic tissue remaining In March 1966 she had an abortion at the 3rd month of gestation and finally she delivered a living girl in May 1968

Of the 21 women with primary sterility 8 conceived, resulting in 4 children, 2 pregnant at the time of writing and 2 abortions. Of the 24 women with secondary sterility 9 conceived, resulting in 7 children and 2 abortions. The period of sterility for the women who conceived was on an average 49 months, and for the women who did not conceive, 56 months.

The prognosis is generally considered very bad after salpingostomy alone, therefore these results have been assessed independently This group includes patients who had bilateral salpingostomy or unilateral salpingostomy on the only remaining tube, irrespective of an accessory operation at the same time. There were 25 patients in this group, 15 with primary and 10 with secondary sterility 13 conceptions occurred in 9 women with the following results (Table III).

Five women had achieved successful pregnancies and 2 were still pregnant at the time of writing. Two patients' conceptions ended in abortion.

Of the 7 women who had previous laparotomies only one conceived as compared with 16 (42 %) of the 38 women who had not had previous operation

Hypospermia was found in 10 husbands. Hypospermia was diagnosed when there were less than 50 mill./ml sperm, or less than 60 % mobile or more than 25 % abnormal forms. Of the women married to these men only 3 conceived compared with 14 of the remaining 35 women.

The number of conceptions in the different age-groups are shown in Table IV

The number of conceptions in relation to the degree of adhesions found at operation was also assessed (Table V)

The first conception occurred from 1 to 65 months after operation, with an average of .5 months. Nine patients conceived in the first year and thereafter there were 1–. conceptions in the following years. The time of observation for the

CONCLUSION

We find that plastic operations on the tubes continue to have their place in the treatment of sterility and that the prognosis is more favourable than usually considered. Also it does not appear to be necessary to use other than the traditional techniques and that steroid and antibiotic treatment are not essential.

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group namely 57 patients who had abnormal hysterographic findings as the only explanation of their sterility and were not treated surgically Nineteen of these 57 patients conceived (Table VI)

Of the non-surgically treated women 23% had intrauterine pregnancies compared with 38% of the treated patients, and it should be added that the latter include the patients with more severe changes. In the non-treated patients there were 40 patients with some anatomical changes, but with patent tubes, whilst the remaining 17 were diagnosed as having tubal occlusion or hydrosalpinx and of these only 3 conceived. In one of these 3 hysterography showed no passage of dye through the tubes, but the hysterography was neither repeated nor was insufflation performed. In the second patient bilateral occlusion was diagnosed, and there was negative insufflation. She became pregnant and aborted in the 4th month, after which insufflation was positive. The last of the 3 patients must be classified as sterile, according to the results of her investigations. Meanwhile she developed a right-sided tubal pregnancy which eventually was treated surgically in conjunction with salpingostomy on the other side. It is evident that there is a high incidence of extra uterine pregnancies in the non-operated cases, as may be expected, but the low incidence of such pregnancies in the operated cases was pleasantly surprising. In a similar comparison Boné Andersen et al (1968) found no difference in the two groups.

We find it surprising that only one of the 5 patients who had exploratory laparatomies conceived. In 3 other patients normal salpinges were found, and none of these conceived suggesting that the cause of sterility in these cases were unknown. It is questionable whether or not the operation results would be better if one found better treatment, for example, for hypospermia, spermagglutination and hostile cervical secretion. Swolin also raised the same question suggesting that there are often other causes which depreciate the results. It is interesting that in Swolin's series, tubal patency was found in 90% of women, but only 22% had intrauterine pregnancies.

In 22 of the 28 women who did not conceive, other reasons were found for a negative result. Four had signs of ovarian dysfunction assessed either by measurement of the basal temperature or by premenstrual endometrial biopsy and in one of these the partner had hypospermia. In 6 others the males had hypospermia, and in 3 of these women there was a history of salpingitis. In 9 of the women there was a history of salpingitis or gonorrhoea, 2 had endometriosis, and one had had a periappendicular abscess.

The series is too small to make an assessment of the prognosis based on different factors, except to say that the prognosis seems to be better in young women, who have not had lower abdominal operations and had few adhesions, whilst the occurrence of previous pregnancies did not seem to influence the prognosis. The length of the sterility did not seem to have much influence on the prognosis.

In assessing the results, it is essential that the period of observation is sufficiently long. Siegler (1964) classifies patients who have not conceived within 2 years after the operation as a failure. In the above series 7 of the 17 pregnancies occurred later than 2 years postoperatively

Any assessment of the prognosis dependent based on operative technique is not possible, because too few salpingolyses and resections have been done, but it is noticeable that the pregnancy frequency for salpingostomy alone is actually the same as for the whole series.

As previously mentioned, no postoperative investigations of tubal patency have been done, and therefore the results should be accepted with some reservation.

When one is able by traditional technique to achieve a rate of 38% of intrauterine pregnancies in those women operated on for sterility one can be a little more optimistic concerning the prognosis than previously It is possible also, that it is not so necessary to consider all other fertility factors to be in order before operating, e.g. mild hypospermia in the partner should hardly be a contraindication for operation It is a recognised fact that reduced fertility in one partner is often partially compensated by increased fertility in the other and the majority of women one considers operating on are women who would in all probability never conceive without operation. Thorough pre-operative investigations are essential, including laparoscopy and, despite traditional technique, surgery should be atraumatic. We do not find any indication for steroid or antibiotic treatment.

Acta Obstet Gynec Scand 50: 117–128 1971

ULTRASTRUCTURE OF THE ANCHORING VILLI AND TROPHOBLASTIC SHELL IN THE SECOND WEEK OF PLACENTATION

Jørgen Falck Larsen and Mogens Knoth

From the Laboratory of Human Reproductive Biology, the Department of Obstetrics A and Gynecology I (Heads: Professor D. Trolle and Dr. Børge Sørensen) and the Institute of Pathological Anatomy (Heads: Professor G. Teilum and Professor E. Jensen), University of Copenhagen, Denmark

Abstract. The fine structure of the anchoring villi and the trophoblastic shell of a human embryo with four somites is described. The ultrastructure of the cytotrophoblast of the proximal part of the anchoring villi is similar to that of the Langhans cells of the free villi. The distal cytotrophoblasts had irregular nuclei and contained more rough endoplasmic reticulum. The cytotrophoblasts of the base of the villi were large and contained large amounts of glycogen.

The basal plate comprised a variety of trophoblastic cells. Most commonly seen was the mononuclear giant cell, which was rich in rough endoplasmic reticulum, glycogen and fibrils. In the periphery of the placental site, the trophoblasts were scattered among the maternal cells. Foetal and maternal cells were often found side by side without any reaction. However the cell membranes of trophoblastic giant cells and those of the uterine gland cells were closely opposed and desmosomes were observed between the foetal and maternal cells. In other places cell membranes between trophoblastic cells and uterine epithelium seemed to disintegrate.

The ultrastructure of the implantation site has been studied in a number of species during the last years (4, 11, 17). Human material from the first weeks after ovulation has been studied with the light microscope only. The material obtained for the light microscopic studies cannot be used for ultrastructural observations as special fixatives and embedding methods are required.

The present investigation deals with the earliest human material studied so far with the electron microscope. It was obtained from the trophoblastic shell of a human embryo with four paired somites having the conception age of approximately — days (6).

The proper implantation has been completed at this stage of development, but the trophoblastic invasion has not been arrested. Therefore, this material gives important information on the human trophoblastic invasion.

The light microscopy and histochemistry of the basal plate was studied by Wislocki & Bennett (20), Wislocki (19), McKay & al. (14), Wislocki & Padykula (21), and Dallenbach-Hellweg (2). Boyd & Hamilton (1) investigated the origin of the cells of the basal plate by tracing the trophoblastic streamers deep into the endometrium.

The fine structure of the anchoring villi from placental sites of higher gestational age has been studied by Enders (3), and Wynn (22) described the basal plate at term.

The ultrastructure of the free chorionic villus has been described in a previous paper (8).

MATERIAL AND METHODS

The embryo was found in a uterus removed at the Copenhagen Municipal Hospital because of precancerous cancer of the cervix. The case history was reported in details by Larsen & Fuchs (13).

Biopsies were taken from the central area and the periphery of the placental site. The tissue was fixed in 1% osmium tetroxide in White's saline (18). It was dehydrated through increasing concentrations of acetone and embedded in Vestopal W. Ultrathin sections were cut on Porter Blum and LKB ultramicrotomes and stained with uranyl acetate and lead citrate. The sections were examined in Siemens and JEOL electron microscopes.

Tissue was fixed for light microscopy in Bouin's solution and embedded in paraffin. However much information was obtained when the one micron-thin sections from the Vestopal blocks were examined in the light microscope. The Vestopal sections used for light microscopy were stained with 1% toluidine blue.

Announcement

The German Society of Endocrinology has awarded the Schoeller Junkmann Preis 1971 instituted by Schering AG to Dr Govind S. Rao Institut für Klinische Biochemie der Universität Bonn, for his publication "Steroidglucuronyltransferasen"

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Professor V N Shirodkar Bombay died on March 3rd 1971 in Doctor s Disease.

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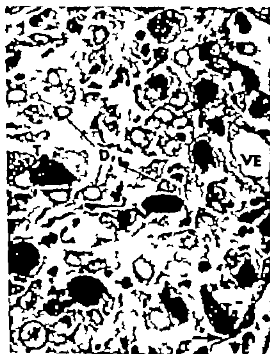
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RESULTS

Light Microscopy

The definitive architecture of the placenta could be recognized The trophoblastic trabeculae have been arranged radially on the surface of the chorionic sac, taking their villous appearance (Fig 1) Some of these, the *anchoring villi* extended all the way from the chorionic sac to the maternal tissue. The anchoring villi appeared cylindroid or conical in shape with diameters ranging from 150 to 200 μ They had an irregular course, were more or less tortuous, and had few branches (Fig 1) Their length varied considerably but no villus was longer than one millimeter

The structure of the *proximal portion of the anchoring villus* was identical with that of the *free chorionic villi, whereas the mesenchymal core* was replaced by cytotrophoblast in the distal portion (Figs. 1 and 2) The syncytial trophoblast covering the Langhans cells of the proximal portion of the villus continued distally until the floor of the intervillous space where syncytial masses entered the basal plate (Figs. 2 and 3) The syncytial trophoblast formed an incomplete layer at the base of the villus, and most of the floor of the intervillous space lacked syncytium (Fig. 2)

The *cytotrophoblastic columns* were formed *by the cytotrophoblasts of the distal portion of* the anchoring villus (Figs. 2 and 3) The proximal columnar cytotrophoblasts were indistinguishable from the Langhans cells of the free villi. The cytotrophoblasts of the most distal portion of the anchoring villus were larger and more irregular in shape (Figs. 2 and 3) Some of these cells stained metachromatically with toluidine blue, probably because of large glycogen deposits (Fig. 3)

The cytotrophoblasts mushroom out from the base of the anchoring villus into the maternal tissue (Fig 3) forming a more or less complete layer of trophoblast, the *trophoblastic shell* This layer consisted of a variety of trophoblastic cells. Most of the trophoblasts were large mononuclear cells, but multinucleated giant cells and syncytial masses were also seen (Figs. 2 and 3) Portions of the syncytial tissue had dense nuclei and large vacuoles.

Trophoblastic cells were observed far into the endometrium. In the periphery the trophoblasts were scattered among the maternal cells, glands, and vessels (Figs. 3 and 4) Thus, a distinct boundary between foetal and maternal tissue has not been established at this stage of placentation.

The *epithelial cells* of the uterine glands were large, cylindroid or cuboidal. The luminar portion of the cells projected into the lumen of the gland. This portion of the cytoplasm stained metachromatically with toluidine blue. Metachromatic secrete was observed in the glands.

The *decidua* of the trophoblastic invasion zone consisted of mononuclear polygonal cells measuring 20–30 μ in diameter (Figs. 3 and 4).

Electron Microscopy

The anchoring villus

The ultrastructure of the *proximal* cytotrophoblast was identical with that of the Langhans cells of the free villi (see Knoth (8)) They were connected with desmosomes and formed a rather compact zone with narrow intercellular spaces. The nuclei of these cytotrophoblasts were regular ovoid. The cytoplasm contained many free ribosomes and rare elements of rough endoplasmic reticulum. The *mitochondria were ovoid with few irregular* and curved cristae.

The *distal* cytotrophoblasts were large, polygonal cells (Fig. 5) The nuclei were more irregular than those of the proximal cytotrophoblasts. Free ribosomes were also common in these cells. However they contained more rough surfaced endoplasmic reticulum than the proximal cytotrophoblasts. Golgi elements were often seen. The mitochondria were globoid or polygonal with few irregular cristae. Glycogen was present in *the form of uniformly dispersed* particles. The cell membrane was irregular and possessed micro-

Figs. 1 and 2 Light micrographs from a paraffin section showing anchoring villi and the basal plate. 100 and 50.

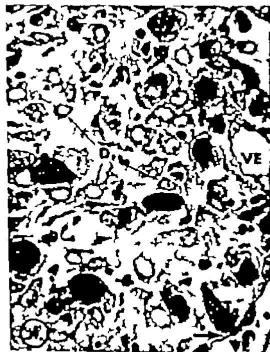
Fig 3 Light micrograph from Vestopal section stained with toluidine blue. Note the basophilia of the trophoblasts. 300.

Fig 4 Light micrograph from a Vestopal section stained with toluidine blue Several trophoblastic giant cells (T_{1-4}) are scattered among the decidual cells (*D*) and between the maternal vessels. The trophoblast are basophilic. The empty spaces represent glycogen accumulations. One of the trophoblasts (T_4) has a very close relation to the maternal vessel (cf. Fig. 7). × 450.


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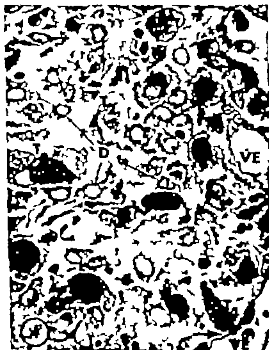
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villi-like processes. The cells were connected with desmosomes.

The intercellular space was wider than that of the proximal portion of columna. This space contained granular medium-electrondense material and fibrin, but blood cells were not observed between the cytotrophoblasts.

The *cytotrophoblasts of the basal portion of the columnae* were large cells with considerable accumulations of glycogen. These cells were ultrastructurally identical with the glycogen-rich type of the basal plate.

The basal plate

The trophoblasts of the basal plate comprised a variety of cell types. Most common was the *large mononuclear cell* (Figs. 6 7 10 14 and 15) The cells of this type were irregular stellate or spindle shaped (Figs. 3 4 and 10) globoid (Fig. 7) or polygonal (Fig. 14) They had large nuclei with irregular nuclear membranes and prominent nucleoli. The cytoplasm contained large amounts of rough surfaced endoplasmic reticulum (Figs. 7 and 14) and Golgi elements (Fig. 6). The mitochondria were of a normal size and contained few cristae (Figs. 6 and 7)

Some of the mononuclear trophoblasts had deposits of glycogen (Figs. 7 and 14) occupying large portions of the cytoplasm. Bundles of fibrils were often found (Figs. 6, 9 and 15)

Stellate osmiophilic inclusions were often seen (*Figs. 7 10, 14 and 16*) These inclusions were probably lipid droplets, but they differed from the lipid found in the epithelial cells (Figs. 14 to 16)

Another type of inclusion consisted of electron dense granular material surrounded by a complete membrane (Figs. 6 and 9)

An unidentified inclusion or organelle (Fig. 8) was found in a few mononuclear giant cells. It consisted of groups of ten to fifty elements, each measuring about 0.5 μ in diameter The elements were disc-shaped and were stacked up as "piles of coins" or rouleaux of erythrocytes. They were concentrated in the cytoplasm in portions which were rich in fine fibrils.

Phagocytosis was observed in some of the giant cells (Fig 10) The phagocytosed material was often found as ghost cells" or portions of cells in the cytoplasm of the trophoblast surrounded by a more or less complete membrane. Some of the phagocytosed cells were erythrocytes.

Syncytial masses of trophoblast—or trophoblastic *giant cells of syncytial type*—were also present in the deep portion of the basal plate. The cytoplasm of this cell type was similar to that of the syncytium of the chorionic villus, but the nuclei were more irregular

The *cytotrophoblasts of the deep part of the basal plate* appeared very active (Fig. 11). They had a very well developed endoplasmic reticulum occupying most of the cytoplasm. However the nuclei had condensation of chromatin under the nuclear membrane and cytoplasmic vacuoles indicated degeneration. Many of these cells were surrounded by large masses of electron-dense, partly fibrillar material—the beginning of the "fibrinoid"

The *decidual cells* of the invasion zone (Figs. 6 and 10) were scattered between the trophoblastic cells. The decidua cells were mononuclear cells, spindle shaped or stellate. Desmosomes were not observed between decidual cells. They were easy to distinguish from the trophoblasts by their regular nuclei and endoplasmic reticulum of a common type. The mitochondria were smaller than those of the trophoblasts, and generally they were more regular in the decidua than in the trophoblast.

The *uterine epithelium* of the invasion zone showed moderate degeneration. These cells were rich in glycogen, often occupying most of the cytoplasm Lipid droplets were often found (Figs. 14 to 16)

Relations between the trophoblast and the maternal tissue

In the present stage of development, a mixture of trophoblastic and maternal cells was present in the basal plate (Fig. 4) The trophoblastic giant cells were found in close contact with the decidual

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Figures 5 to 16 are electron micrographs.

Fig. 5 Portions of cytotrophoblasts from the distal part of columna. Note the simple mitochondria (*m*). Cytoplasmic processes (*p*) extend into the intercellular spaces. 8 000.

Fig. 6. Trophoblastic giant cell (top) in close contact with decidual cell. Note the difference in the shape of the nuclei and in the contents of the cytoplasms. No fibrillar material is found between the cells. The cytoplasm of the trophoblast contains much fibrillar material (*f*) and inclusions (*i*). 18 000.

cells only separated by a narrow intercellular space (Fig. 6) At most of these places no reaction has been observed between the two cells. However some of the decidual cells seem to be covered by an electron dense material simulating a basement membrane (Fig. 12)

The maternal cells seem to yield to the trophoblasts, which seem to "push" on the maternal cells. This results in displacement of gland cells (Fig. 14) or causes an impression of the cell membrane of the adjacent maternal cell (Figs. 6 and 7)

In many places, the trophoblast was found in very close apposition to maternal cells. This was the case in a uterine gland where a giant cell was found "pushing" the epithelial cells into the lumen of the gland (Fig. 14) The basement membrane of the epithelium was interrupted at the place of trophoblastic invasion. The cell membranes of the trophoblastic giant cell and the gland cells ran parallel only separated by a narrow intercellular space of same width as the space between the individual gland cells. The trophoblastic giant cells and the uterine epithelium were connected with desmosomes in some of the glands (Fig 15)

The placental site was rich in lymphocytes which were often found in close relation to trophoblastic cells (Fig. 13)

Trophoblastic cells were found in many maternal vessels, but no trophoblast was observed in the process of penetrating a maternal vessel However many trophoblasts were observed in close relation to the maternal endothelium (Fig. 7). The cell membrane of the trophoblasts was drawn out in numerous blunt processes.

Cytoplasmic fusion between trophoblast and epithelium was observed in some of the glands (Fig 16). The cell membranes were interrupted in these places and it was difficult to tell where the trophoblast ended and the uterine epithelium started.

DISCUSSION

The present study of the ultrastructure of the anchoring villi confirms the observations of many light microscopists. *The three types of cytotrophoblasts of the columna correspond with those demonstrated by Wislocki & Bennett (10) in their histochemical survey* The proximal cytotrophoblasts are histochemically and ultrastructurally indistinguishable from the Langhans cells. As shown in a previous study (8), the Langhans cells contribute to the syncytium by assimilation of their cytoplasm and nuclei therein. The syncytial lining of the basal part of the intervillous space may be produced in a similar manner from the proximal columnar cytotrophoblasts. However it might as well simply be a remnant of the peripheral syncytium through which the outgrowth *of the cytotrophoblastic columns has taken place.*

The differentiation of the columnar trophoblast increases with the distance from the mesenchymal core. The ultrastructure of the distal portion confirms the histochemical demonstration of glycogen and ribonucleoprotein (14). Hertig (7) distinguishes between indigenous and ingested glycogen. The first type is found in the cytotrophoblast, the chorionic mesoderm, and the germ disc, whereas the ingested glycogen is located as large rounded masses, "lollipops" in the syncytiotrophoblast. Although Hertig (7) based his description on a 13-day-old embryo, it seems most likely that the glycogen—accumulated or not—in the cytotrophoblast of the anchoring villi is of the indigenous type.

The large glycogen deposits in the trophoblastic cells may provide an energy supply for anaerobic metabolism as the distance between the cells and the intervillous space increases. Glycogen was not found in the trophoblasts of the deepest portion of the basal plate. These trophoblasts were surrounded by "fibrinoid" and showed indications of degeneration (Fig 11) The arrest of the trophoblastic invasion may be explained by exhaustion of glycogen combined with the isolation from the blood supply because of fibrinoid coating.

The abundance of rough surfaced endoplasmic reticulum in the trophoblast of the distal portion of the columna and the basal plate indicates protein synthesis. *Such protein may be either proteo*lytic enzymes used in the invasion of the endo-

Fig 7. Trophoblastic giant cell (left) in close contact with the endothelium of a maternal vessel (right). The cell membrane is irregular with many small processes (*P*). Note the stellate lipid droplets (*L*) and the glycogen accumulation (*gh*). The *basement membrane* (*be*) of the endothelium is still intact. 15 000.

Fig 8. Unidentified cytoplasmic organelle. 15 000.

Fig 9 Portion of a trophoblastic giant cell containing a bundle of fibrils (*f*), rough endoplasmic reticulum (*r*), and an inclusion with granular material (*i*). 30 000.


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cells only separated by a narrow intercellular space (Fig. 6) At most of these places no reaction has been observed between the two cells. However some of the decidual cells seem to be covered by an electron dense material simulating a basement membrane (Fig. 12)

The maternal cells seem to yield to the trophoblasts, which seem to "push" on the maternal cells. This results in displacement of gland cells (Fig. 14) or causes an impression of the cell membrane of the adjacent maternal cell (Figs 6 and 7)

In many places, the trophoblast was found in very close apposition to maternal cells. This was the case in a uterine gland, where a giant cell was found pushing the epithelial cells into the lumen of the gland (Fig 14) The basement membrane of the epithelium was interrupted at the place of trophoblastic invasion The cell membranes of the trophoblastic giant cell and the gland cells ran parallel only separated by a narrow intercellular space of same width as the space between the individual gland cells. The trophoblastic giant cells and the uterine epithelium were connected with desmosomes in some of the glands (Fig. 15)

The placental site was rich in lymphocytes which were often found in close relation to trophoblastic cells (Fig. 13)

Trophoblastic cells were found in many maternal vessels, but no trophoblast was observed in the process of penetrating a maternal vessel However many trophoblasts were observed in close relation to the maternal endothelium (Fig 7) The cell membrane of the trophoblasts was drawn out in numerous blunt processes.

Cytoplasmic fusion between trophoblast and epithelium was observed in some of the glands (Fig. 16) The cell membranes were interrupted in these places and it was difficult to tell where the trophoblast ended and the uterine epithelium started.

DISCUSSION

The present study of the ultrastructure of the anchoring villi confirms the observations of many light microscopists. The three types of cytotrophoblasts of the columna correspond with those demonstrated by Wislocki & Bennett (20) in their histochemical survey The proximal cytotrophoblasts are histochemically and ultrastructurally indistinguishable from the Langhans cells. As shown in a previous study (8), the Langhans cells contribute to the syncytium by assimilation of their cytoplasm and nuclei therein. The syncytial lining of the basal part of the intervillous space may be produced in a similar manner from the proximal columnar cytotrophoblasts. However it might as well simply be a remnant of the peripheral syncytium through which the outgrowth of the cytotrophoblastic columns has taken place.

The differentiation of the columnar trophoblast increases with the distance from the mesenchymal core. The ultrastructure of the distal portion confirms the histochemical demonstration of glycogen and ribonucleoprotein (14) Hertig (7) distinguishes between indigenous and ingested glycogen. The first type is found in the cytotrophoblast, the chorionic mesoderm, and the germ disc, whereas the ingested glycogen is located as large rounded masses, lollipops in the syncytiotrophoblast. Although Hertig (7) based his description on a 13-day-old embryo it seems most likely that the glycogen—accumulated or not—in the cytotrophoblast of the anchoring villi is of the *indigenous type*

The large glycogen deposits in the trophoblastic cells may provide an energy supply for anaerobic metabolism as the distance between the cells and the intervillous space increases. Glycogen was not found in the trophoblasts of the deepest portion of the basal plate These trophoblasts were surrounded by fibrinoid" and showed indications of degeneration (Fig. 11) The arrest of the trophoblastic invasion may be explained by exhaustion of glycogen combined with the isolation from the blood supply because of fibrinoid coating.

The abundance of rough surfaced endoplasmic reticulum in the trophoblast of the distal portion of the columna and the basal plate indicates protein synthesis. Such protein may be either proteolytic enzymes used in the invasion of the endo-

Fig 7 Trophoblastic giant cell (left) in close contact with the endothelium of a maternal vessel (right). The cell membrane is irregular with many small processes (*P*). Note the stellate lipid droplets (*L*) and the glycogen accumulation (*gly*). The basement membrane (*bm*) of the endothelium is still intact. 15 000.

Fig 8 Unidentified cytoplasmic organelle 35 000.

Fig 9 Portion of trophoblastic giant cell containing a bundle of fibrils (*f*), rough endoplasmic reticulum (*er*), and an inclusion with granular material (*i*). 30 000.


er
m



metrium or it may be contributing to the fibrinoid, which begins to appear at this stage of development. The recent studies by Moe (15) show that the fibrinoid is composed by fibrin, cellular debris, glycogen-like granules, and granular masses. The latter was suggested to be a mixture of secretory products from the cells and material derived from the maternal blood (15)

It is possible that these trophoblasts produce hormones, also Ultrastructurally they are very similar to the cytotrophoblastic cells of choriocarcinoma, *which in tissue culture produced large* amounts of chorionic gonadotropin (10).

A strange cytoplasmic structure was observed in some of the trophoblastic giant cells (Fig. 8) It consisted of a large number of discoid elements. This structure was previously observed in choriocarcinoma (9) and chorioadenoma des ruens (16) These structures may be remnants of cell membranes or desmosomes.

Enders (3) described a fibrous type of cytotrophoblast in the basal plate. Many of the trophoblasts in this study contained large bundles of fibrils (Figs. 6 9 15 and 16). Tonofibrils could be found in almost all trophoblastic cells of the basal plate in variable amount, and we have failed to find a specific fibrous type at this stage of development.

The present study has shown that the trophoblastic invasion has not been arrested on the 22nd day *after the ovulation On the contrary* trophoblastic cells were scattered among the maternal cells and were found deep into the endometrium. Wynn (22) found it difficult to distinguish between *trophoblasts and decidual cells in the basal* plate at term In the present study the maternal and foetal cells differed in the size and shape of the nuclei as well as in the endoplasmic reticulum. Wynn (22) *stated that at term the human* trophoblastic and decidual cells were invariably separated by fibrinoid or necrotic debris. or both This type of material was found in few places only *in our specimen in which the foetal and* maternal cells were in direct contact.

Although the specimen represents a late stage of the trophoblastic invasion, some of the features observed correspond with earlier stages in implantation of other species. Potts (17) described a "stage of attachment" during the implantation in the mouse. This is characterized by a reduction of microvilli *on both sides*. The junctional zone becomes "a serpentine contour with clumpsy villi and irregular cytoplasmic projections The next step is the *adhesion stage* in which the juxtaposed membranes run parallel for most of their course separated by a 20–25 mμ gap (17). Fig. 14 of the present study shows a similar situation in which the cell membrane of a trophoblastic giant cell is closely *apposed to the cell membranes of* four gland cells.

Further Potts (17) observed that the trophoblast became attached to the maternal cells with *junctional complexes. This phenomenon has later* been found during the implantation in man species (5) and Larsen et al. (12) demonstrated desmosomes between human trophoblasts and hamster liver cells in transplanted choriocarcinoma. In the present study this stage could be found, also. Some of the trophoblastic giant cells have established desmosomes with uterine gland cells (Fig. 15).

A fusion between the cytoplasms of maternal cells and trophoblasts have been observed in few places (Fig. 16) A similar cytoplasmic fusion takes place in an early stage of the implantation in the rabbit (11) No degenerative changes were found in the uterine epithelium during the fusion in the rabbit, but in the present study the glandular cells appeared to be degenerating (Fig. 16). Thus, the cytoplasmic fusion observed in this study may be the first step of phagocytosis of the *uterine epithelium by the trophoblast.*

Many trophoblasts were found in close contact with the maternal endothelium (Fig 7) or in the lumen of the vessels, but places with actual pene-

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Fig 10 Trophoblastic giant cell with phagocytosed material (*Ph*). Note the very irregular nucleus (*VI*) and the prominent nucleolus ( ) In left lower corner a decidual cell with a regular nucleus (*AD*) th smooth nuclear membrane. The difference between the endoplasmic reticulum of the two cells is also demonstrated. 000.

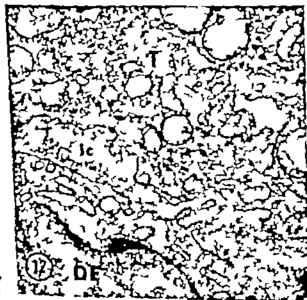
Fig 11 Trophoblast in the deep part of the basal plate. The cell has a very developed rough surfaced endoplasmic reticulum ( ) Th. chromatin is concentrated below the nuclear membrane The cell is completely surrounded by electron-dense—partly fibrillar—substance the "fibrinoid" (*FI*). 3 000.

Fig 1 Fibrillar substance between a trophoblastic cell (top) and decidual cell. 20 000

Fig 13 A lymphocyte in close relation to a trophoblast. Note that the cytoplasmic process from the lymphocyte is "pushed" into the trophoblast (between arrows). × 15 000.


Ic
n
NT
10


NT
n
11


T
Ic
12
DE


NL
13

Fig 14 A trophoblastic giant cell has penetrated the basement membrane (*BE*) of the epithelium of a uterine gland. The cell membrane of the giant cell and those of the gland cells (*Uter epithel* and *Ut Ep*) are closely apposed (*arrows*). 500.

tration of a vessel were not observed. It is possible that the invasion of the vessels occurs in areas of degeneration However in a preliminary study of a human implantation site on the 25th day of the menstrual cycle, we have observed trophoblasts side by side with viable endothelium. The cytoplasm of the trophoblasts adjacent to maternal vessels possessed blunt processes (Fig. 7) Similar processes were observed during the implantation in the rabbit (11)

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Explanation of Figures

AV anchoring villus.
be basement membrane of the maternal endothelium.
BE, *basement membrane of the glandular epithelium*.
BP basal plate
d, desmosome.
D decidual cell.

Fig 15 A trophoblastic giant cell (*left*) in close relation to uterine epithelium. Note the difference between the cytoplasms of the two types of cells. *Insert:* an area indicated in the large figure. A desmosome (*d*) connects the trophoblast with the epithelial cell. 8 000. Insert, 28 000.

Fig 16 Portion of trophoblastic giant cell (*top*) in contact with the uterine epithelium. The cell membranes have disappeared in many places, and continuity is found between the cytoplasms of the two cells (*arrows*). 16 000.

DC distal portion of columna.
DS degenerating syncytial masses
EN endometrium.
er rough surfaced endoplasmic reticulum.
f cytoplasmic fibrils.
FI fibrinoid.
f free ribosomes.
FI free villus.
G Golgi apparatus.
GL uterine gland.
gly glycogen.
i cytoplasmic inclusion.
ic intercellular space
IS intervillous space
L lipid droplet in trophoblast.
ll lipid droplet in uterine epithelium.
m mitochondrion.
n nucleolus.
ND nucleus of decidual cell.
NE nucleus of endothelial cell.
NG nucleus of glandular cell.
NL nucleus of lymphocyte.
NT nucleus of trophoblast.
PC proximal portion of columna.
Ph phagocytosed material.
SY syncytial masses.
T trophoblast.
Ut Ep and *Uter epith I* uterine epithelium.
VE lumen of maternal vessel.

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A NEW METHOD FOR STUDYING THE MOTILITY OF THE FALLOPIAN TUBES

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Abstract A new method for investigating the motility of the Fallopian tubes is described. A few droplets of iodized oil are injected into one tube and their pattern of behaviour is studied by X-ray fluorography using both television picture and cine-film.

It was found that the behaviour of the droplets appears to follow specific pattern, but that some variations occur.

Technically the technique is relatively simple and does not cause much discomfort to the patient. It permits reasonable assessment of the peristaltic activity of the tubes to be made.

The motility of the Fallopian tubes has long been subject of great interest. Westman (1926, 1929 1937) was the first to investigate this phenomenon in the rabbit. Following Westman's experimental work the peristaltic activity of the tubes has been studied extensively in the human being by hysterosalpingography laparoscopy and at laparotomy (Pincsohn, 1924 Kok, 1945 Daniel, Mitescu, Soimaru and Georgescu, 1937 Rubin, 1938, 1947 Doyle, 1953 1956 Beclere, 1955 Rozin and Schwartz, 1965 Rovalo Gimenez, 1967 Blanco, Rozada, Remedio, Hendriks and Alvarez, 1970). Laparoscopy and laparotomy are surgical procedures which have to be carried out under general anaesthesia. This probably interferes with the peristaltic activity of the tubes. Moreover it is difficult to believe that healthy women will be willing to undergo these procedures merely to help with physiological research.

During the past few years new method of studying the motility of the Fallopian tubes has been developed and assessed in this department. Technically it is relatively simple and involves, at most, only slight discomfort for the patient. It requires no anaesthesia and only minimal interference with the tubes by instruments. It is therefore hoped that this description of the technique will pro e to be of interest.

CASE MATERIAL

The method as used on 28 omen hose ages ranged from 30 to 45 years. The endometrium was found to be in the secretory phase in 10 omen and in the proliferative phase in the other

All had previously had normal pregnancies and salpingography carried out in conjunction with this investigation, showed normal tubes in every case.

More than half of the omen ere volunteers. The other women sought medical advice because of secondary infertility of long duration or because of obscure pain.

METHOD

Three cannulae of different lengths and diameters are used, each fitting accurately into the next in size (Fig. 1). These consisted of

(i) straight 50 cm long polyethylene tube, No. Pe 30(IC), its inner and outer diameters being 0.5 mm and 1.0 mm respectively Its tip is bevelled and its proximal end is fitted ith a needle to facilitate connection,

(ii) radiopaque red Oedman catheter (ROC), 40 cm long, its inner and outer diameters being 1.2 mm and 2.2 mm respectively The distal 4 cm of the catheter are curved through 80° the other end is fitted with collar for attachment of an adapter ith tap

(iii) straight polyethylene tube, No. Pe 320 (OC), 30 cm long, the outer and inner diameters being 2.69 mm and 3.50 mm respectively

If the ROC is introduced into the OC so that the curved end protrudes for only few mm, the former is almost straight (Fig. 2). If the distal end of the ROC protrudes 4 cm the terminal part will bend 80° (Fig. 3). Thus, the distal 4 cm of the ROC can be adjusted to form curve of from 0° to 80° depending on the length of this part of the catheter hich protrudes from

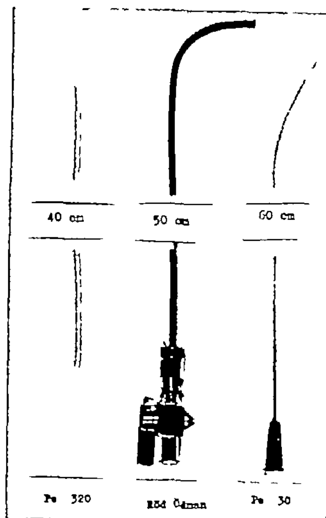


Fig 1 Instrument for catheterisation of the Fallopian tube. Only the proximal and distal ends of the catheters are shown. The figures indicate their lengths in cm. *Pe 320* = outer catheter *Pe 30* = inner catheter

the OC. This enables the shape of the distal end of the ROC to be adjusted so that it corresponds with that of the uterine cavity The introduction of the instrument into the uterus is thereby facilitated, particularly if it is markedly anteflexed or retroflexed. Another advantage of the flexibility of the distal end of the ROC is that its shape can be modified further after its introduction into the uterine cavity if it should be required for passing the instrument through the uterine cornu into the tubal ostium.

The examination did not require any premedication of the patient. The patient was placed on the X-ray couch in the lithotomy position. The cervix was grasped with volsella, the external os was cleansed, and the position of the uterus was determined, using a silver sound. The ROC was then introduced into the OC, the curve of the former was adjusted so that it corresponded with the shape of the uterine cavity and the instrument was introduced through the cervix. The patient was now placed supine and the instrument was turned towards the uterine cornu until the tip of the ROC faced the tubal ostium, controlling the manoeuvre with television fluoroscopy

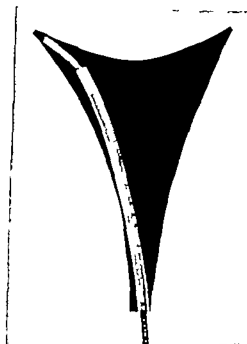


Fig Diagrammatic representation of the uterine cavity showing the position of the instrument if the uterine cornu is directed obliquely upwards.

As the curve of the ROC can be increased or decreased as required it is usually quite easy to introduce the instrument until its tip lies in the tubal ostium.

If the anatomy of the uterine cornu could not be determined with confidence a few cc of water-soluble opaque medium (angiografin, Schering AG Berlin) were injected through the ROC into the uterine cavity which could then be seen on the television screen and exact positioning of the ROC was possible. Thereafter 1 cc



Fig 3 Diagrammatic representation of the uterine cavity showing the position of the instrument if the uterine cornu is directed laterally

of angiografin was injected into the tubal ostium. If the position of the instrument was correct only the tube opacified. Thereafter the opaque medium as washed out of the tube by slowly injecting physiological saline solution. The IC as then introduced into the ROC as far as possible. In the majority of cases the tip was in the intramural part of the tube but occasionally it extended as far as the isthmus. In 2 cases the catheter could not be introduced at all. In order to check the position of the IC in the tube further 1 cc of angiografin was injected. As knowledge of the position, length and shape of the tube is of major importance for the rest of the procedure radiograph was taken to confirm the information obtained from the television screen (Fig. 4). Thereafter the opaque medium as washed out of the tube at the same way as on the previous occasion. A few drops of iodized oil ere then injected into the medial part of the tube. The instrument was ithdrawn and the volsella ere removed.

The behaviour of the droplets as studied by X-ray television. As pictures of optimal quality are required for such study drastic coning (X-ray beam collimation) as used; the size of the field of the skin of that part of the abdomen of the patient nearest to the image intensifier's input screen ranged from 1 1 cm to 2 cm. In order to enable minute details of the behaviour of the droplets to be studied the image was magnified 2.

The movement of the droplets varied but generally it as jerky. The length of the latent intervals also showed variations (Fig. 5).

Continuous observation by X-ray television for a prolonged period of time would have involved excessive radiation doses to the ovaries. The investigation was therefore carried out intermittently over period of 1 hour the individual observation times ranging from 5 to 10 sec. The intervals between the observation periods



Fig. 4. Radiograph of the right tube showing the inner catheter introduced about 6 cm into the tube. The tip seen to be at the isthmic portion of the tube (*unmarked arrow*). The tip of the radiopaque Oedman catheter shown to lie close to the tubal ostium (*arrow marked a*).

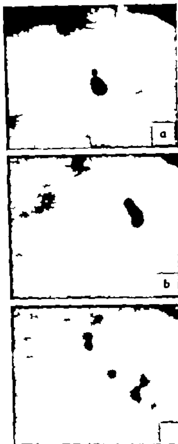


Fig. 5. Radiographs of few droplets of oil in the isthmic portion of the tube (34-year-old woman who attended for secondary infertility. The endometrium was in the proliferative phase). (*a*) Taken 5 min after the injection of the droplets into the tubal isthmus. They are seen to have migrated towards the ampulla and to have coalesced, forming large and small droplet which lie close together. (*b*) Taken 30 min after (*a*). The droplets are seen to have migrated towards the fimbriated end of the tube. They have coalesced, forming single, oblong droplet. (*c*) Taken 55 min after (*a*). The oblong droplet has divided into 6 droplets; 3 lie adjacent to the fimbriated end of the tube. Immediately after this radiograph was taken 3 of the droplets were seen to be expelled into the abdominal cavity.

depended on the speed at hich the changes in the movement and shape of the droplets occurred, but generally ranged from 10 to 60 sec.

For further study minute details of the pattern of behaviour of the droplets were recorded on cine-film for periods ranging from 10 to 30 sec. The speed at which the droplets moved and at hich the changes in their shape and position occurred, usually permitted the use of low frame speeds. In the initial stages of this investigation higher frame speeds ere used, i.e. to 48 frames/

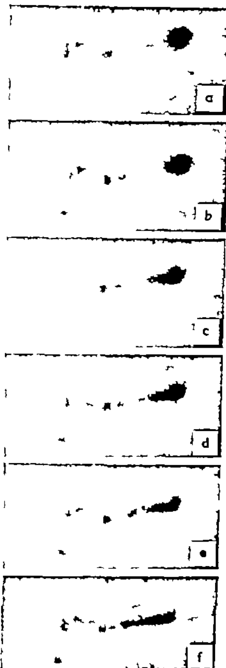


Fig 6 Ciné-film of 6 droplets of oil in the isthmic portion of the right tube (39 year-old volunteer; the endometrium was in the secretory phase). Frame rates 6 frames second. Films *a–f* show the behaviour of the droplets during 1 sec. The largest droplet is seen to be *in the medial part of the isthmus, the others being in* the lateral part. The largest droplet has assumed an oblong shape and has moved towards the small droplets which have not changed their position.

second. As it was found that the use of higher frame speeds did not afford any information of greater value *only* 3 to 6 frames/second were taken subsequently (Fig. 6).

The droplets were seen to move to and fro along the tubal lumen, to divide *into two or more parts, to* coalesce and to change their shape and relative position before they were expelled *into the abdominal cavity*.

The position and shape of the droplets, as portrayed on the television screen, were recorded diagrammatically using the following technique. Plastic sheeting was placed on the television screen *and the contours of the droplets* were drawn.

The use of the video-tape recorder will enable us to abandon diagrammatic representation of the droplets and probably also the *use of cinéfluorography*.

The X-ray television equipment used and the radiation dose to the ovaries

Early on in this investigation several radiographs of the tube were taken but it was soon found that only one film was required for checking the information obtained from the television screen.

A study of tubal motility by the method described makes heavy demands on the X-ray television system used. The resolution power of the apparatus should be sufficiently high to enable minute details to be seen and *it should be sufficiently sensitive to assure pictures of* optimum quality and definition even with the use of relatively small radiation doses.

It was found that of the available X-ray television systems, *the SAAB XTV 70 (SAAB Medical Division, Linköping, Sweden)* meets these demands. Another advantage of the apparatus is that it is fitted with a 35 mm ciné-camera which permits the use of low frame rates *such as* 1 *to 3 frames sec with highly* stable frame rate.

With this system the exposure rate applied by fluoroscopy as measured at the image intensifier's input screen, *usually is 10 mR hour. It* should be mentioned, however that the field used on the occasion when the above radiation dose was calculated, was much larger than that used in this investigation. It is technically difficult to measure the exposure rate with such small fields as were used in this investigation. With cinéfluorography, the exposure rate at the *image intensifier's input screen* was 10 μR/frame.

It may be assumed that the technique described involves the following radiation doses:

(i) the single radiograph taken involves a radiation dose of about 0.1 R to the ovaries

(ii) a six-minute exposure time for fluoroscopy results in an exposure rate to the ovaries equivalent to that from one conventional radiograph. As the combined exposure times for fluoroscopy were on average 1 min, the radiation dose is equivalent to that from 5 radiographs, i.e. 0.5 R,

(iii) twenty-five-second exposure time for cinefluorography taking 3 frames second involves an exposure rate to the ovaries equivalent to that from one conventional *radiograph*.

In about half of the cases cinéfluorography was not used. In the other cases ciné films were taken for *10 to* 60 sec, the radiation dose to the ovaries being on average 0.2 R.

The total radiation dose to the ovaries from the technique described appears to be on average 1 R, i.e. about the same *dose* which these organs receive during

hysterosalpingography It should be borne in mind, however that with the technique described only one ovary is exposed, the irradiation of the other ovary is considerably less.

COMMENT

The main object of this paper was to describe a new method for investigating tubal motility A few droplets of iodized oil are injected into one tube and their behaviour is studied by X-ray television and cinefluorography

Admittedly the true conditions are probably not revealed by this technique but a fair idea of the peristaltic activity of the tubes is obtained. From the observations made it is concluded that the behaviour of the droplets follows a specific pattern but that variations occur

Tubal motility has previously been studied radiographically by hysterosalpingography This examination involves filling of both tubes and the entire uterine cavity Opacification of these organs causes their dilatation. Hysterography has shown that the motility of the uterus decreases or ceases following dilatation of the uterine cavity It may therefore reasonably be assumed that this also applies to the motility of the tubes. As the tube is not dilated in the technique described it may be assumed that the presence of a few droplets of oil in its lumen interferes only slightly with its motility if at all. The irritant effect of iodized oil on the mucosa is negligible

The possible relationship of the variations in the pattern of behaviour of the droplets to the phases of the menstrual cycle and the possibl influence of drugs will be the subjects of a future paper

It should be mentioned, that the technique described also permitted recording of intratubal pressure.

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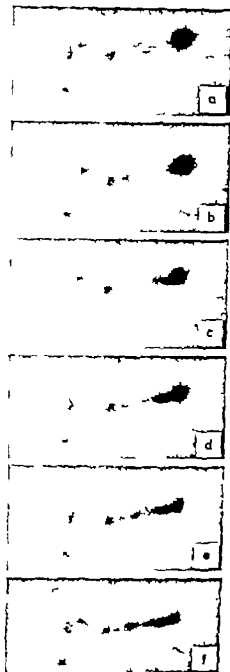


Fig. 6. Ciné film of 6 droplets of oil in the isthmic portion of the right tube (39-year-old volunteer; the endometrium was in the secretory phase). Frame rate: 6 frames/second. Films *a–f* show the behaviour of the droplets during 1 sec. The largest droplet is seen to be in the medial part of the isthmus, the others being in the lateral part. The largest droplet has assumed an oblong shape and has moved towards the small droplets which have not changed their position.

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Early on in this investigation several radiographs of the tube were taken but it was soon found that only one film was required for checking the information obtained from the television screen.

A study of tubal motility by the method described makes heavy demands on the X-ray television system used. The resolution power of the apparatus should be sufficiently high to enable minute details to be seen and it should be sufficiently sensitive to assure pictures of optimum quality and definition even with the use of relatively small radiation doses.

It was found that of the available X-ray television systems, the SAAB XTV 70 (SAAB Medical Division, Linköping, Sweden) meets these demands. Another advantage of the apparatus is that it is fitted with a 35 mm ciné-camera which permits the use of low frame rates such as to 3 frames/sec with highly stable frame rate.

With this system the exposure rate applied by fluoroscopy as measured at the image intensifier's input screen, usually is 10 mR/hour. It should be mentioned, however, that the field used on the occasion when the above radiation dose was calculated, was much larger than that used in this investigation. It is technically difficult to measure the exposure rate with such small fields as were used in this investigation. With cinéfluorography the exposure rate at the image intensifier's input screen was 10 μR/frame.

It may be assumed that the technique described involves the following radiation doses.

(i) the single radiograph taken involves a radiation dose of about 0.. R to the ovaries,

(ii) a -minute exposure time for fluoroscopy results in an exposure rate to the ovaries equivalent to that from conventional radiograph. As the combined exposure times for fluoroscopy were on average 15 min, the radiation dose is equivalent to that from 5 radiographs, i.e. 0.5 R,

(iii) twenty-five-second exposure time for cinéfluorography taking 3 frames/second involves an exposure rate to the ovaries equivalent to that from conventional radiograph.

In about half of the cases cinéfluorography was not used. In the other cases ciné-films were taken for 10 to 60 sec, the radiation dose to the ovaries being on average 0.2 R.

The total radiation dose to the ovaries from the technique described appears to be on average 1 R, about the same dose which these organs receive during

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INHIBITION OF OXYTOCIN-INDUCED UTERINE ACTIVITY IN MIDPREGNANCY BY COMBINED ADRENERGIC α-BLOCKADE AND β-STIMULATION

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Abstract. Arrest of imminent premature labour by β-adrenergic stimulation often fails, due to undesirable side effects or lack of effectiveness. Inhibition of oxytocin release from the neurohypophysis by ethanol also has disadvantages.

α-adrenergic stimulation in pregnant and non-pregnant women increases uterine contractions, while blocking of α-receptors in non-pregnant patients has been shown to reduce uterine activity. A combination of adrenergic α-blockade followed by β-stimulation therefore might be more effective than β-stimulation alone in the treatment of premature labour.

This combined method was used in 6 women at 16–20 weeks of pregnancy. The response to oxytocin-induced uterine contractions was measured.

In mid-pregnancy blockade of α-receptors did not reduce oxytocin-induced activity. β-stimulation reduced the uterine activity; this reduction seemed to be independent of α-blockade.

The combination of α-blockade and β-stimulation did not seem to be superior to β-stimulation alone.

From a clinical point of view there is a considerable demand for an effective, simple and safe method for inhibition of uterine contractions, especially during the last weeks of pregnancy.

Clinical experiments (2, 10) give support to the hypothesis that α- and β-adrenergic receptors do exist in the myometrium. In general, the effect on α-receptors is excitatory and that on β-receptors is inhibitory. β-adrenergic stimulation, especially with isoxsuprine, is used in the treatment of imminent premature labour. Undesirable side effects and limited potency restrict its acceptance as an ideal myometrial inhibitor.

Inhibition of oxytocin release with ethanol (3) also has its limitations. An ethanol-intoxicated premature child may be borne. Moreover ethanol infusion may possibly induce uterine contractions. Increased uterine activity accompanied by a rise in secretion of vasopressin has been shown to occur in non-pregnant women during ethanol-induced nausea (1). The occasional failure of ethanol infusion to stop premature labour when patients become nauseated may be due to a similar increase in neurohypophyseal secretion. We have witnessed this combination of nausea and increased uterine activity during ethanol infusion in late pregnancy on two occasions (Fig. 1).

Wansbrough et al. (10) found increased uterine activity during α-stimulation with noradrenaline in pregnant and non-pregnant women. This response was inhibited by α-blocking agents. α-blockade also reduced spontaneous activity in non-pregnant patients. During labour a corresponding reduction of spontaneous activity did not take place; however, the dose given was rather small, and only 2 cases were tested.

We therefore thought it worthwhile to test a combination of adrenergic receptor α-blockade and β-stimulation. The activity still present in the myometrium after tolerable doses of β-stimulating drugs might be caused by endogenous α-receptor stimulation.

α-blocking agents administered in late pregnancy give tachycardia and increased cardiac stroke volume in the mother due to a fall in peripheral vascular resistance (5). These side effects might be aggravated by β-stimulation and be detrimental to the foetus. Therefore patients admitted in mid-pregnancy for therapeutic abortion were chosen for the experiments.

Book Reviews

Erik Rydberg: *Stereoscopic X-ray in Obstetrics.* An Atlas and a *Clinical Study* Pp. 170, 19 figs., 13 tables and 40 stereograms in a separate folder including a binocle for stereoscopic viewing. Universitetsforlaget i Aarhus, Denmark, 1969 Price US $49 —

During the years 1956–1962 Professor Rydberg made stereoscopic X-ray examinations in bout 530 cases of delivery using a special pparatus, allowing a pair of stereoscopic anterior posterior iews of the pelvis and abdomen to be taken within a few seconds, thus excluding blurring and distortion, due to movements of fetus or mother Based upon this big material an excellent description is given of the theory and physiology of binocular vision, the mechanism of engagement, the descent and delivery of the fetus. A description is also *given of the anatomy and classification f the pelvis and its soft* tissues.

The material presented in the stereoscopic atlas is unique and extremely well prepared for teaching purposes. The atlas is so arranged, that it will stand open on a table for easy viewing, with the binocle enclosed. This out standing work ought to be vailable in every school of obstetrics and midwifery

I S

F E. Szontagh. *The Mechanism of Action of Oral Progestogens.* 176 pp., 57 figs., 47 tables. Akadémiai Kiadó, Budapest 1970.

The work deals exclusively with the effects of semisynthetic oral progestogens from the point of view of reproduction. The ffects of compounds with nor-steroid structure ha e been investigated as well as the properties of progesterone derivates with an acetylated hydroxyl group in the 17-alpha position and with the substitution of the H-atom on C-6. The no -steroid group seemed to the author to be more significant because of their ovulation blocking effect. The mode of action by which ovulation is blocked has not been clarified in every detail. The author provides some experimental evidence for the existence of hypothalamo-pituitary internal gonadotrophin feedback.

Animal experiments as well as clinical investigations have been done in order t clear up the mechanism of the contraceptive effect. Many different progestogens has been checked. But the results of most animal experiments cannot be pplied immediately to human beings. The doses used on animals were ery large For xample 4 mg lynestrenol daily for 1 days has been used for the inhibition of ovulation in rats. Combined treatment with oestrogen and progestogen in doses of 4 mg lynestrenol and 30 μg mestranol has the same effect.

For the clinical studies assay methods have been used which are now more or less out of date. This is a pity because the results may therefore be questionable. In spite of this fact it seems as if a dose of 5–10 mg lynestrenol daily decreases the output of total gonadotrophins in the urine of women. 17-alpha-hydroxyprogesterone has no effect upon the output of total gonadotrophins in the urine. The effect of 19-norsteroids is enhanced when oestrogens are added. An interesting observation is that the inhibitory effect of lynestrenol upon the output of gonadotrophins from the pituitary depends not only *upon the dose given but also upon the hormonal status* of the woman.

The author also investigates whether there is a direct ovarian site of action but the problem cannot be regarded as solved from his experiments. The effect of different contraceptive preparations on the motility of the human fallopian tube in vitro is also studied and he states that the motility is markedly inhibited. Both the amplitude and frequency of the contractions are depressed by progestogens. The effect of combined preparations (lynestrenol + mestranol) upon the endometrium and the cervical mucus *has also been studied. Earlier observations are confirmed.*

The book concludes with a chapter on the therapeutic uses of progestogens. I cannot agree with all of the author's indications for their use. The "rebound" effect after the use of combined preparations is very questionable which the cases of long-standing amenorrhoea after the use of contraceptive pills confirm. The pregnancy maintaining effect of progestogens is also very much under discussion.

The author reports a marked stimulating effect upon hormone excretion during pregnancy following injections of allyloestrenol and methyloestrenolone. He points out that norsteroids increase the activity of trophoblastic epithelium in different ways but concludes that the exact way in which norsteroids may effect the function of the placenta can be elucidated only by *further* investigations.

M. F

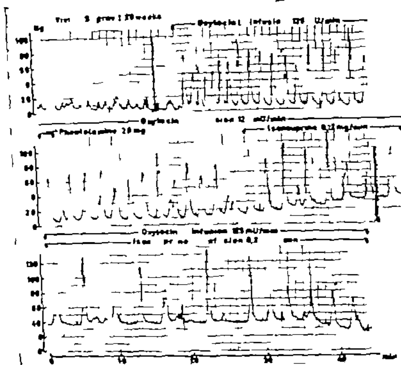


Fig. 2. Pressure curve from an abridged experiment (patient not included in the series). Vertical lines before start of oxytocin infusion and during isoxsuprine infusion 0.12 mg/min represent calibration. Increasing resting tonus during experiment was not constant finding. Recording paper speed 1 cm/min.

dose was limited by pallor and some restlessness. The sensitivity to oxytocin showed considerable variation, unrelated to basal uterine activity.

After α-blockade the uterine activity in cases 1 and 4 was unchanged (Table III). Cases 2 and 3 showed decreases of 33 and 18% respectively while in patients 5 and 6 uterine activity increased by 15 and 124% respectively. Thus the response varied within wide limits although on an average, α-blockade caused no change in uterine activity. In each patient the response was constant throughout the experiment. Frequency dropped on average from 7¼ to 6¼ contractions per 10 min. Uterine activity per contraction increased, somewhat more when calculated in APA than in MU (Table IV). This increase after phentolamine is not statistically significant, irrespective of the method of measurement.

β-adrenergic stimulation with isoxsuprine re-

Table II. *Spontaneous and oxytocin induced uterine activity*

Case no.	Spontaneous activity (Montevideo units)	Induced activity: mU oxytocin infused per min	Induced activity: Montevideo units
1	2	160	159
2	56	100	442
3	31	200	332
4	0	160	336
5	16	240	348
6	73	160	149
Average	29	170	294

Table III. *Uterine activity per 10 min during oxytocin infusion, before and after phentolamine 20 mg intravenously*

Case no.	Montevideo units: Before phentolamine	Montevideo units: After phentolamine	APA: Before phentolamine	APA: After phentolamine
1	159	167	5	5.3
2	442	297	17.7	17.1
3	332	272	15.1	10.9
4	334	313	11.9	12.7
5	348	399	14.7	16.6
6	149	334	8.7	17.3
Average	294	297	12.2	13.3

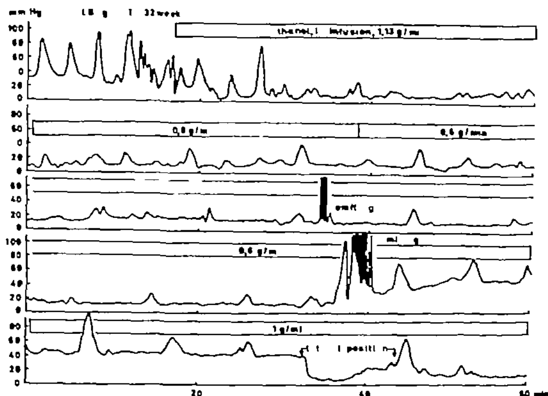


Fig 1 Ethanol infusion, starting dose 1 13 g/min, f fecti ely inhibits uterine contractions. First short spell of nausea and omiting does not change uterine activity but during the second attack of nausea uterine activity increases to labour strength, necessitating rather heavy dosage of ethanol for supression. Recording with Malmstrom external tocograph. Cervix unchanged during recording period, –3 cm open, unruptured membranes.

PATIENTS AND METHODS

Six patients were studied, and relevant clinical data are given in Table I The patient were reassured to avoid psychic stress or fear N premedication was given, the anterior abdominal wall was infiltrated with 10 ml 1 % Xylocain® in each patient. An open end vinyl catheter with many side holes was threaded percutaneously into the amniotic cavity through a thick cannula and connected by closed water system to an Elema Schönander pressure transducer EMT 34 Amniotic fluid loss was avoided. The pressure curv was traced on a Siemens Kompensograph III ink recorder

When stability had been reached fter puncture, basal uterine activity was measured for t least 30 min (Fig)

Table I *Clinical data of patients*

Case no.	Age (years)	Duration of pregnancy (weeks)	Earlier pregnancies
1	20	17	0
2	15	20	0
3	19	18	0
4	36	16	1
5	19	17	0
6	20	16	0
Average	20¹/	17	

Uterine contractions were then induced by intravenous oxytocin infusion. Recording was continued for at least 20 min after a fairly high and stable activity had been reached.

Phentolamine (Roehtin® Ciba) 70 mg was then injected slowly intra enously and activity registered for 25 min; the pressure curv for the last 20 min was used for calculations. This constant and short period for phentolamine acting alone was chosen to ensure a reliable α-blockade during the last experimental phase.

After this period, β receptor stimulation was started with infusion of isoxsuprine (Cardilan® Ferrosan) in the first 4 cases, and DU 1220 (Ritodrine, Philips-Duphar) in cases 5 and 6 Increasing doses were given T or three 10 min periods ere measured t each dose level

Uterine activity was calculated in Montevideo units (MU), and active pressure area (APA), measured with a Haff planimeter in cm² 10 mm, neglecting resting tonus. Values given in the tables are a erages of the measured 10 min periods. Blood pressure and pulse rate were measured every 5 min after phentolamine injection The experiments as well as the following therapeutic abortions induced by hypertonic saline were without complications in all patients.

RESULTS

The dose of oxytocin given varied from 100 to 240 mU per min (Table II) In cases 1 and 6 the

sponding observations have been made in postpartum dogs (7). Adrenergic nervous system stimulation inhibited uterine activity the inhibition being blocked by phentolamine.

The β-adrenergic receptor is thought to be identical with the enzyme adenyl cyclase (6), which increases the production of adenosine 3′ 5′-monophosphate (cyclic AMP) from ATP The β-adrenergic stimulators exert their effect by stimulating this enzyme (9). As phentolamine does not seem to interfere with the response to β-adrenergic stimulation, it is less likely that phentolamine accomplishes any direct myometrial effect via the cyclic AMP system.

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Table IV *Uterine activity per contraction during oxytocin infusion, before and after phentolamine 20 mg intravenously*

Case no	Montevideo units		APA	
	Before phentolamine	After phentolamine	Before phentolamine	After phentolamine
1	20	28	0.66	0.73
2	64	60	2.60	3.31
3	59	60	2.71	2.39
4	32	33	1 12	1.34
5	50	64	2.10	2.64
6	27	6[illegible]	1.55	3 00
Average	42	51	1 79	2.24

Table V *Oxytocin induced activity before and during isoxsuprine infusion (phentolamine given 25 min before start of infusion)*

Case no	Oxytocin infusion		Isoxsuprine infusion					
			0 12–0.16 mg/min		0.24 mg/min		0.48 mg/min	
	MU	APA	MU	APA	MU	APA	MU	APA
1	159	5	103	3 6				
2	442	17 7	263	12.4	177	8.6		
3	332	15.1	315	15 4	275	11 6	268	9.5
4	336	11.9	290	12	131	6.2		

duced the uterine activity in proportion to the dose given (Table V) The 2 patients given DU 21220 reacted similarly (Table VI). Patient 6 showed the greatest increase in uterine activity after phentolamine possibly due to a high catecholamine level. In spite of this, she tolerated the highest dose of DU 21220 given, 1 mg/min.

Resting tonus and regularity of contractions were measured but gave no information of interest.

SIDE EFFECTS

Phentolamine caused no recognizable side effects, in spite of a relatively high dosage. Hypotension and tachycardia accompanied isoxsuprine infusion and limited increasing doses. Compared with other patients given only isoxsuprine, it is our impression that these side effects were to some extent aggravated by the preceding α-adrenergic blockade. On the other hand DU 21220 resulted in no side effects.

COMMENTS

On average, α-blockade did not influence uterine activity confirming earlier findings in patients near term (10) In the rabbit, myometrial content of noradrenalin and adrenergic nerve endings increase in early pregnancy and decrease toward term (8) If there is a similar variation in the human myometrium, and the nerve endings mediate α-adrenergic impulses, the lack of effect of phentolamine implies that this decrease takes place during the first 4 months of human pregnancy

In an unpublished study of 3 patients 15–18 weeks pregnant, we gave isoxsuprine as in the present series, without influencing the α-receptors pharmacologically Oxytocin-induced activity was reduced 22–56% Considering the dosage, this decrease, and the decrease reported in patients near term (4) are of the same magnitude as in the present series, showing that phentolamine does not influence the myometrial response to isoxsuprine. Phentolamine must be considered as a pure α-blocking drug, with regard to its effect on the myometrium.

In cases 5 and 6 α-blockade increased uterine activity suggesting that the α-receptors in these patients were under endogenous suppressive influence in contrast to the general rule. Corr-

Table VI *Oxytocin induced activity before and during infusion of DU 21220 (phentolamine given 25 min before start of infusion)*

Case no.	Oxytocin infusion		Infusion of DU-21220											
			0.1 mg/min		0.2 mg/min		0.3 mg/min		0 5 mg/min		0.75 mg/min		1 mg/min	
	MU	APA	MU	APA	MU	APA	MU	APA	MU	APA	MU	APA	MU	APA
5	348	14 7	278	10.4	170	5.6	127	4 5						
6	149	8 7					42	11.8	181	7 4	141	6.3	125	4.6

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CYTOLOGICAL DIAGNOSIS OF ENDOMETRIAL DISORDERS WITH A BRUSH TECHNIQUE

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Abstract. Sixty-two patients with various endometrial disorders or normal endometrium were investigated by cervical and vaginal smears, endometrial smears and thorough curettage, and the results obtained by the three methods were compared. The endometrial smears were taken by using a modified "brush technique". This technique makes it possible to take endometrial samples on frequently repeated occasions, without anesthesia and without dilatation of the cervix.

The results of this study indicate that the "brush technique" yields cytological material satisfactory for the diagnosis of some endometrial disorders.

Using endometrial material obtained by the "brush technique" planimetric measurements of the cell nuclei were carried out in four groups of five subjects each representing four different types of endometrial activity such as proliferative, early secretory, late secretory and atrophic. An analysis of variance revealed that the cell nuclei were highly significantly different at each of the four phases studied. It is concluded that the cytological material obtained by the brush technique is suitable for objective cell analysis e.g. planimetric measurements, cytophotometric studies etc. It is suggested that the brush technique also offers excellent possibilities for studying the metabolism of isolated endometrial cells.

Routine vaginal cytology is widely used in gynaecological practice but is of limited value in the diagnosis of endometrial disorders. Under certain conditions when the patients are bleeding from the uterus, endometrial cells are desquamated and accumulated in the vaginal pool. A cytological examination of such accumulated material may reveal malignant cells originating from an endometrial carcinoma. It has been reported that the rate of detection of malignant cells in the vaginal pool is approximately 50 to 70% in cases of endometrial carcinoma (Boschann, 1958 Burns et al., 1950, Koss & Durfee, 1962 Reagan & Ng, 1965) However for the purpose of dating the endometrium during the menstrual cycle cytological examination of the cellular material accumulated in the vaginal pool is of little, if any value. Undoubtedly direct sampling would give more valuable information about the endometrium than a smear from the accumulated cellular material in the upper part of vagina.

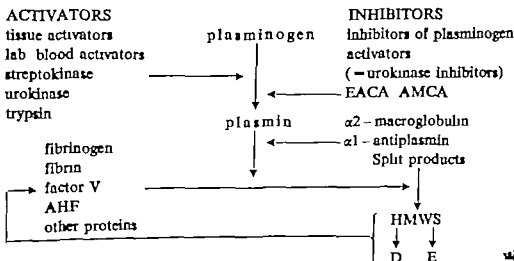
Since all diagnostic cytology is based upon the interpretation of minute intracellular details, a proper collection and preservation of the endometrial material is of outmost importance. Various techniques have been employed in order to obtain well preserved cytological material from the endometrium. Aspiration of endometrial material by introducing a metal cannula into the uterine cavity was used by Cary (1943) and similar endometrial aspiration methods with small modifications have been described by several other workers (Abramson & Driscoll, 1966 Clyman, 1955 Delleplane, 1958 Haour 1958 Hecht, 1956 Nuovo, 1958 Rascoe, 1963 Terzano, 1958). The endometrial lavage technique has been described by Morton et al. (1959). They injected physiological sterile saline into the uterine cavity The lavage fluid was then aspirated from the vaginal pool, centrifuged and prepared for smears. A similar technique was used by Lubke (1965). A sponge-swab technique has been described by Diamond et al. (1952) A small cellulose or gelatin sponge was introduced into the uterine cavity and the endometrial cells were collected on the sponge. Similar methods with some modifications were described by Bickenbach & Soost (1958) and by Chatfield & Watson (1970). Brush methods have also been applied. Ayre (1955) used brush of natural bristles enclosed

Gynaecologists

Menorrhagia may be caused by an increase in local fibrinolytic activity Cyklokapron reduces menorrhagic haemorrhages by an average of 50 %

Women with average menstrual blood losses of over 80 ml have higher concentrations of plasminogen activators in the endometrium than those with lower blood losses The resultant increase in local fibrinolytic activity is inhibited by Cyklokapron The recommended dosage of Cyklokapron in menorrhagia is 1 g 3–6 times daily for 3–6 days With a dosage of 3 g daily Nilsson and Rybo noted reductions in bleeding of 38 % compared with control cycles With twice this dosage bleeding was reduced by 51 %. None of the 36 patients participating in the trial were obliged to discontinue treatment as a result of side-effects.

Reference NILSSON L., RYBO G Treatment of menorrhagia with an antifibrinolytic agent tranexamic acid (AMCA) A double blind investigation Acta Obstet. Gynecol Scand. 46 (1967) p 572



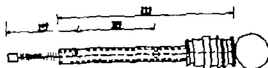


Fig. 2. The polyethylene catheter enclosing the nylon brush.

follows. A 25 cm long polyethylene catheter with diameter of 3.5 mm was used. The catheter enclosed thin nylon brush with diameter of 2 mm. The brush could be pushed through the catheter as illustrated in Fig. 2.

The polyethylene catheter enclosing the thin brush was introduced into the uterine cavity without any cervical dilatation and the nylon brush was pushed forward 2 to 3 cm. The endometrial cells were then collected on the nylon bristles by some circular movements. The brush was drawn back in the protecting polyethylene catheter after which the instrument was removed. This procedure was adopted in order to avoid contamination with material from the cervical canal as the brush was being removed. The cells collected on the nylon bristles were spread out on glass slide and immediately fixed by aerosol of polyethylene glycols and isopropanol ("Spray-Cyte"). They were stained by the Papanicolaou method.

Following the collection of the cytological material the patients were subjected to dilatation and curettage. In all cases thorough curettage was performed for diagnostic reasons and the material was prepared for histo-pathological examination by embedding in paraffin and by staining the sections in haematoxylin-eosin. The histological material was examined for the presence of malignancy and the dating of the endometrium was done according to the principles described by Noyes, Hertig & Rock (1950).

A preliminary dating of the endometrium was performed on cytological basis simultaneously with an examination of the material for the presence of malignant cells. The dating of the endometrial cells was mainly based upon two cytological criteria, the varying chromatin pattern of the endometrial cell nuclei and the variation in size of the nuclei. In addition, some cytological changes of the cytoplasm of the glandular cells during the menstrual cycle were taken into consideration. During the early secretory phase the cytoplasm of these cells was often loaded by secretory products, whereas the glandular cells originating from an early proliferative endometrium or an atrophic endometrium revealed small amount of cytoplasm usually with cyanophilic staining reaction.

In order to investigate the reliability of one of the criteria used, planimetric measurement of the nuclear area of the endometrial cells was performed in limited number of patients. For this study 20 patients were selected, representing four different stages of endometrial activity. The selection of the patients was based upon the histo-pathological findings and all cases of atypia and malignancy were excluded. The corresponding cytological material was submitted to the planimetric measurements and the study was performed as a "double blind" test. A planimeter (type Aristo 1130-L) was used and microphotographs of the endometrial cells were used for the planimetric measurements. In each patient the areas of 25 endometrial cell nuclei were estimated and there was approximately an equal number of glandular cells and stroma cells. The mean area was calculated for each patient and statistical analysis was performed for the four groups investigated.

RESULTS

The findings of the cytological material obtained from the endometrium by the brush technique described were compared with the histo-pathological diagnosis of the curettage material. The results of this comparison are shown in Table II.

The cytological diagnoses reported in this table were all based upon microscopical evaluation of isolated endometrial cells and cell clusters. Cytological material composed of endometrial cells displaying some variation in nuclear size and showing a coarse, irregular chromatin pattern was considered as originating from a proliferative endometrium (Fig. 3).

Endometrial cells and cell clusters with uniform nuclei showing a more finely dispersed chromatin were considered as characteristic of the secretory endometrium. Some of these cells occasionally

Table II. *Correlation between cytology and histology*

Histological diagnosis	Cytological diagnosis					
	Total no of cases	Proliferative activity	Secretory activity	Atrophic	Atypia	Carcinoma
Proliferative activity	27	26	1	—	—	—
Secretory activity	15	2	13	—	—	—
Atrophic mucosa	12	1	—	11	—	—
Glandular hyperplasia	5	5	—	—	—	—
Adenocarcinoma	3	—	—	—	—	3
Total	62					

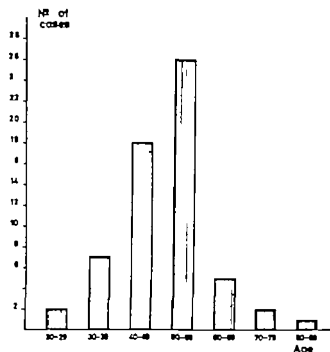


Fig. 1. Age distribution among the 62 patients investigated.

in a polyethylene tube and a similar method was described by Boschann (1957, 1958), who used a nylon brush enclosed in a metal cannula. A modification of this endometrial brush technique was introduced by Fox et al. (1962). They used a brush without protecting tube and the endometrial material was collected on the bristles. The cells were rinsed in physiological saline and centrifuged and the deposit was used for cytological and histological examination. An endometrial brush similar to that described by Fox et al. (1962) was used by Johnsson & Stormby (1968).

All methods described above have been designed for the detection of malignancy in the endometrium. However the brush technique seems to yield material also suitable for dating of the endometrium based upon cytological criteria (Boschann 1957, 1958).

The dating of the endometrium is a prerequisite not only for the diagnosis of endometrial disorders but also for the evaluation of the effect of various hormones upon the endometrium. It has been reported that the effect of various types of oral contraceptives is reflected by morphological changes in the endometrium (Czernobilsky et al., 1969; Maqueo-Topete et al. 1964; Starup, 1967). The use of intra-uterine devices also seems to induce endometrial changes (Wynn, 1967). The increasing use of oral contraceptives as well as intra-uterine devices accentuated the need for a simple method of obtaining endometrial material for diagnosis, and the brush technique seems to be most suitable for this purpose.

The present investigation was designed to evaluate the advantages and limitations of the endometrial cytology for dating the endometrium, by using a technique which is as painless as possible for the patient but which simultaneously yields endometrial cells suitable for cytological diagnosis. A modification of the brush technique described by other authors (Ayre, 1955; Boschann, 1957, 1958) was considered useful for obtaining satisfactory material.

Table I

Clinical diagnosis	Number of patients
Dysfunctional uterine bleeding	19
Postmenopausal bleeding	9
Myoma	10
Ovarian cyst	2
Cervical polyp	1
Primary dysmenorrhoea	1
Chronic salpingitis	1
Suspected TB	6
Total	49
Normal cases	13

MATERIAL AND METHODS

The total number of women examined during the period of the study was 62. Most of these patients were premenopausal, the age distribution is shown in Fig. 1.

The majority of the patients had a history of intermenstrual or postmenopausal bleedings. However some of the women were admitted to the hospital because of a suspicion of tuberculous infection in the genital tract. The control series consisted of 13 patients without any known disorder of the endometrium. The distribution of the case material by clinical diagnosis is presented in Table I.

All patients were examined by routine cervical and vaginal smears. In addition, endometrial smears and curettings were taken. The cervical and vaginal smears were taken with a wooden spatula and the cytological material from the cervix and the vagina was immediately fixed by aerosol of polyethylene glycols and isopropanol ("Spray-Cyte", Clay-Adams Inc., New York). The smears were stained by the Papanicolaou method and they were examined for the presence of normal and abnormal endometrial cells.

The technique used for the endometrial smear was as



Fig. 5. Group of endometrial cells originating from an atrophic mucosa. Note the poor amount of cytoplasm of the stroma cells as well as of the glandular cells. ×100.

no atypical changes could be recognized. Malignant cells were found in 3 out of 3 cases with histologically verified carcinoma.

The cervical and vaginal smears revealed some groups of well preserved endometrial cells without any atypia in one of the five cases with histologically verified glandular hyperplasia. No endometrial cells were found by this method in any other cases. In the three cases of histologically verified carcinoma no atypical or malignant cells

Table III. *Areas of endometrial cell nuclei given in* μ^2 *with S.D.*

Twenty women at 4 stages of endometrial activity. Each figure is the mean of 25 individual measurements

Proliferative activity	Early secretory activity	Secretory activity	Atrophic mucosa
44.64 10.59	62.25 ± 9.03	39.91 ± 6.95	27.53 ± 6.47
41.91 7.22	51.09 ± 13.70	28.02 ± 7.83	36.70 ± 10.42
34.97 ± 8.93	50.59 ± 14.17	33.73 ± 7.61	32.49 ± 11.50
49.10 ± 7.99	68.94 ± 11.22	43.38 ± 8.72	24.30 ± 5.04
47.12 13.27	57.04 ± 9.64	31.25 ± 7.04	38.19 ± 7.74
Mean value 43.55	57.98	35.66	31.84

Table IV. *Analysis of variance of the planimetric study of the nuclear area*

Twenty patients at 4 stages of endometrial activity

Source of variation	S.S. (Sum of squares)	D.f. (Degrees of freedom)	M.S. (Mean of F squares)	(F-value)
Diagnostic groups	136 517.4	3	45 505.8	194.2
Patients	178 371.8	19	9 387.9	40.1
Patients within same diagnostic groups	41 854.4	4	10 463.6	44.7**
Residual (within patients)		480	234.3	
Total		499	—	

** P < 0.001

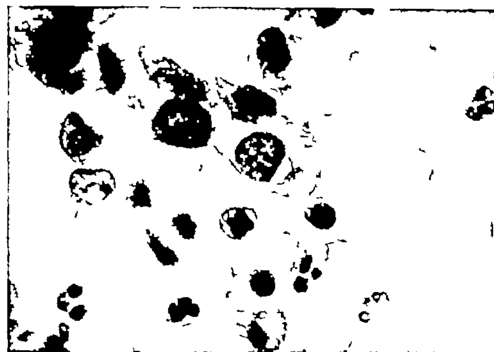


Fig. 3. Endometrial cells obtained by the "brush" technique. Note the well preserved cytoplasm and the coarse chromatin characteristic for the endometrial cells during the proliferative stage. × 350.

displayed a cytoplasm loaded with secretory products (Fig. 4).

The cells obtained from an inactive endometrium revealed small uniform nuclei with a compact chromatin structure and a small amount of cytoplasm (Fig. 5).

The malignant cells were diagnosed according to the cytological criteria used for malignancy (see Koss, 1968).

As shown in Table II the cytological diagnosis was in agreement with the histo-pathological diagnosis in 26 out of 27 cases in early and late proliferative stage and in 13 out of 15 cases with a histologically verified secretory endometrium. In patients with an atrophic endometrium the cytological diagnosis of an inactive endometrium was correct in 11 out of 12 cases. As expected, the cytological diagnosis indicated proliferative activity of the endometrium in the 5 cases with histologically verified glandular hyperplasia and



Fig. 4. Endometrial cells obtained from an endometrium with fully developed secretory activity. Note the cytoplasm of the glandular cells, which is loaded by secretory products. × 400.

tients representing four different types of endometrial activity As revealed in Tables IV and V the analyses of variance showed a highly significant variation between the cell populations. These results indicate that the variation in size of the endometrial cell nuclei can be used as one of the reliable parameters of the endometrial activity Wied et al. (1968) have used the taxonomic intracellular analytic system (TICAS) in order to demonstrate how computer assisted evaluation of various types of cells can lead to the derivation of objective diagnostic criteria. They also analysed aspirated uterine glandular cells by estimating the relative area of the nuclei and of the cytoplasm in normal endometrial cells as well as in cells originating from atypical hyperplasia (Wied et al., 1969) and adenocarcinoma (Wied et al 1969 *b*) in the uterus. However in the studies quoted above, the TICAS assessment of endometrial cells was not applied to the discrimination of endometrial cells during the menstrual cycle. In the present study the endometrial cell nuclei showed a variation in size during the menstrual cycle. A peak in the increase of the nuclear area was found in the early secretory endometrium, when the mean area was estimated to 57.98 μ This peak was statistically highly significant when compared with the values obtained at the proliferative stage as well as when compared with the results of the late secretory phase and the atrophic endometrium. However the highly significant increase in nuclear size of the early secretory stage must obviously be preceded and followed by a stage of development when the sizes of endometrial cell nuclei are identical. This indicates that there must exist a stage in the proliferative endometrium which can not by this criterion be distinguished from that occurring in the late secretory phase. Therefore in order to make a differential diagnosis between the proliferative stage and the fully developed secretory phase of the endometrium, a planimetric assessment can be useful only when combined with another significant parameter Although no objective estimation of the chromatin pattern of the endometrial nuclei has been done in the present study it seems likely that the varying chromatin pattern during the menstrual cycle can be used as such a significant parameter

A variation in the size of endometrial cell nuclei—estimated in the stromal cells as well as in the glandular cells during the menstrual cycle—has also been reported in other planimetric studies (Boschann, 1957 1958 Witt, 1963) Boschann (1957 1958) found an increase of the nuclear area of the stromal cells from 35 μ^2 to 52 μ^2 during the first 13 days of the menstrual cycle and described a shrinkage of the stromal cell nuclei during the fully developed secretory stage. Whether or not this variation in size of the endometrial cell nuclei during the menstrual cycle is correlated with some variation in DNA-synthesis (Vokaer et al., 1953) or to cyclical changes in the metabolism of the nuclei remains to be investigated.

The atrophic endometrium was characterized by a nuclear area significantly smaller than the average value of any diagnostic group investigated during the menstrual cycle. The statistical assessment of the differences between the various diagnostic groups is shown in Table V

From the data of the present study it can be concluded that the "brush technique" as described above yields endometrial material satisfactory for a cytological evaluation of some endometrial disorders. For the diagnosis of endocrine disorders of the endometrium, histological examination of curettings is superior to conventional cytological evaluation of endometrial cells. However it should be emphasized that the cytological method as described in the present study can be used in situations where a biopsy is not feasible. The endometrial smear can also be repeated frequently without discomfort to the patient by using the "brush technique as described above. Furthermore, the cellular material obtained has proved to be suitable also for objective cell analysis. This method of obtaining isolated, well preserved endometrial cells in a simple way may therefore increase the possibility of studying the intracellular metabolism of isolated endometrial cells, by various methods and under different conditions, e.g. following the use of oral contraceptives. Such studies are now in progress.

ACKNOWLEDGEMENTS

We are indebted to Dr P Petrus for valuable discussions on statistical problems and for assistance in computational work.

The expenses of this investigation were defrayed by Research Grants from the Swedish Medical Research Council, the Ford Foundation and the Swedish International Development Authority

Table V *Comparison between the diagnostic groups by the method of orthogonal contrasts*

Twenty patients in 4 stages of endometrial activity

Diagnostic groups	F-value
(E.S.) (P + S. + A.)	457.5
(A.) (P + E.S. + S.)	200.8
(P) (E.S.)	144.6
(P.) (S.)	43..
(E.S.) (S.)	345.7

Critical F value for $P < 0.001 = 10.83$
P = Proliferative activity
E.S. = Early secretory activity
S. = Secretory activity
A. = Atrophic mucosa.

were recognized in the cervical and vaginal smears.

In an attempt to base the cytological diagnosis on objective rather than subjective criteria, a planimetric study was carried out by measuring the variation in the nuclear area of the endometrial cells. The results of this study are shown in Table III.

In the proliferative endometrium the mean area was 43.55 μ^2 During the early secretory stage there was an increase in the nuclear size and the mean area was 57.98 μ^2 A fully developed secretory endometrium displayed a mean area of 35 66 μ^2 per cell nucleus and the mean area of the nuclei originating from an atrophic endometrial mucosa was 31 84 μ^2

The analysis of variance performed in this planimetric study displayed a statistically highly significant variation among all diagnostic groups ($P < 0.001$) As expected, a significant individual variation also existed among patients as shown in Table IV

The four diagnostic groups were compared by the method of the orthogonal contrasts. Also in this analysis highly significant differences ($P < 0.001$) were found between the various diagnostic groups as shown in Table V

DISCUSSION

In order to diagnose various disorders of the endometrium in situations where a biopsy is not feasible it is desirable to find a method which yields endometrial material suitable for cytological analysis without discomfort to the patient. The type of brush used in the present study enables a direct sampling from the endometrium without anaesthesia. Due to the small diameter of the instrument it can be introduced into the uterine cavity without any dilatation of the cervical canal, and contamination of cellular material by cells and discharge from the cervix and the vagina is avoided by the use of the protecting polyethylene catheter Due to the simple method of collecting the cellular material samples of the endometrium can be obtained frequently without discomfort to the patient, which facilitates the following of the cytological changes in the endometrium on several occasions during the same cycle.

The results of the endometrial smears were compared with the cervical and vaginal smears and the endometrial currettage specimens. For the diagnosis of malignant changes in the endometrium the limited value of the routine cervical and vaginal smear has been reconfirmed. In the present study none of the 3 cases of endometrial carcinoma could be diagnosed by examining the cervical and vaginal smears. The direct sampling of endometrial cells by using the brush technique described in the present study revealed malignant cells in all 3 cases where the diagnosis of carcinoma was verified by histological examination. However in spite of the high detection rate of malignancy in the present study the series is too small to allow a valid statistical analysis. Whether or not the brush technique, as described in the present study yields endometrial material suitable for a valid diagnosis of malignant changes remains to be further investigated.

The findings of the endometrial smears were also compared with the currettage material in respect of the dating of the endometrium. Only a few studies have previously been devoted to the dating of the endometrium by cytological methods. Most of these studies have reported nuclear and cytoplasmic changes, which could be related to cyclic changes of the endometrium (Ferrera, 1960 Horava et al. 1961 Peters, 1957 Smolka, 1958) In the present study the comparison between the cytological diagnosis and the histological findings showed very little discrepancy as indicated in Table II. The cytological diagnoses in these cases were all based upon subjective evaluation of the isolated endometrial cells and cell clusters.

Planimetric measurements of the endometrial cell nuclei were performed in four groups of pa-

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CHANGES IN UTERINE VOLUME FOLLOWING THE INTRA AMNIOTIC INJECTION OF HYPERTONIC SALINE

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From the Department of Obstetrics and Gynaecology (Head Professor Lauri Rauramo), University of Turku, Turku, Finland

Abstract A 30% increase in uterine volume during the initial 3 hours after intra-amniotic saline injection has been calculated from 53 amniograms in 8 midtrimester patients

The evolution of uterine activity and abortion, following the intra-amniotic injection of hyper tonic saline has been explained by two effects of the hypertonicity of the liquor namely increased uterine volume and decreased progesterone support for the pregnant uterus (Csapo, 1966) A marked progesterone withdrawal during the instillation abortion time has been documented (Csapo et al., 1969) but the increase in volume of the midtrimester uterus is still debated (King et al 1964 Gochberg & Reid, 1966 Wagner 1966, Goodlin et al., 1968) It was of interest, therefore, to re-examine the correlation between the early changes in uterine olume and myometrial activity (Pulkkinen & Kivikoski, 1969), for recent clinical trials showed a better quantitativ and temporal relationship between the in creased ratio uterine volume/progesterone (V/P) and evolution of uterine activity than between decrease in progesterone alone and activity (Csapo et al., 1970)

MATERIAL AND METHODS

A group of 8 patients were admitted to hospital for legal abortion and sterilization at 16 eeks of pregnancy

By transabdominal puncture removed 25 ml amniotic fluid (AF) and replaced it ith 25 ml Conray-300® contrast medium, so as to obtain measure of the resting uterine volume However these attempts were unsuccessful because of the poor mixing of the contrast medium ith AF

In the final study, therefore, we removed as much AF as possible (under television control), averaging 264 ml and replaced it with a mixture of 180 ml 23% NaCl and 30 ml Conray-300® (Fig 1 a, b, c). Using spot camera technique (to limit the radiation dose to that of routine pelvimetry) antero-posterior amniograms were made under strictly constant conditions at 0, 30, 60, 120, 180, 240 and 360 min following the instillation of saline + Conray® (Fig. 1 c, d). The patients were supplied with 2 000 ml water, to be taken by mouth during period of 4 hours. A radiologist outlined the uterine shadow hich was then measured by nurse, using planimeter.

The accuracy of the method has been determined by injecting five times mixture of 90 ml 0 9% NaCl and 10 ml Conray-300® into 2 cases. This treatment only resulted in an area increase of 0.017 cm²/ml, on the average. I 6 cases amniograms were also taken at 0 time and 60 sec later so as to determine the reliability of repeated measurements before the hypertonic saline has had time to have its effect. Table I illustrates that the average difference between two subsequent measurements of the uterine area at one minute intervals is only 0.5 cm² e.g. 3% Therefore, changes in area greater than this amount (0 5 cm² 3%) were considered valid.

RESULTS

Table II illustrates the observed changes in the uterine area of the 8 study patients. The measurements were made during the initial 6 hours following the instillation of hypertonic saline. All patients showed an increase in uterine areas in 3 hours, averaging 1 4 cm² In patient 1 an ex cessive increase occurred abruptly t 4 hours, followed by spontaneous rupture of the membranes.

Using a calibration curve, we converted the area increase to volume increase. At 3 hours following the intra-amniotic instillation of 20% NaCl we calculated a volume increase of 30%

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CHANGES IN UTERINE VOLUME FOLLOWING THE INTRA AMNIOTIC INJECTION OF HYPERTONIC SALINE

Martti O. Pulkkinen and Asko Kivikoski

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The accuracy of the method has been determined by injecting five times mixture of 90 ml 0.9% NaCl and 10 ml Conray-300® into 2 cases. This treatment only resulted in an area increase of 0 017 cm^2/ml, on the average. In 6 cases amniograms were also taken at 0 time and 60 sec later so as to determine the reliability of repeated measurements before the hypertonic saline has had time to have its effect. Table I illustrates that the average difference between two subsequent measurements of the uterine area at one minute intervals is only 0.5 cm^2 e.g. 3%. Therefore, changes in area greater than this amount (0 5 cm^2 3%) were considered valid.

RESULTS

Table II illustrates the observed changes in the uterine area of the 8 study patients. The measurements were made during the initial 6 hours following the instillation of hypertonic saline. All patients showed an increase in uterine areas in 3 hours, averaging 1 4 cm^2 In patient 1 an excessive increase occurred abruptly at 4 hours, followed by spontaneous rupture of the membranes.

Using calibration curve, we converted the area increase to a volume increase At 3 hours following the intra-amniotic instillation of 20% NaCl w calculated a volume increase of 30%



Fig 1 Amniograms after removal of amniotic fluid (*a*–*b*), at 0 ( ) and at 360 (*d*) minutes following the instillation of 180 ml 23% N Cl + 30 ml Conray 300®

Table I. *Twelve amniograms, 60 sec intervals, after hypertonic saline injection*

Hrs after 20% NaCl	Amniograms (cm² at 0 sec)	At 60 sec
1	16.0	16.3
1	19.9	19.6
2	9.0	10.6
3	11.7	11.8
4	18.1	18.5
6	20.9	20.6
Mean	15.6	16.2

Table II. *Uterine area in antero-posterior amniograms after hypertonic saline injection* (change, in cm²)

Case no	Hrs after injection 1/2	1	2	3	4	6
1	—	1.7	−1.2	0.8	11.5	—
2	−1.2	1.3	0.8	0.2	0.1	3.4
3	1.7	2.0	0.1	2.6	1.0	3.4
4	2.0	1.5	1.6	1.9	2.4	3.3
5	0.9	−0.6	−0.5	0.1	−0.2	1.6
6	2.0	3.5	4.1	4.9	—	3.8
	0.9	1.1	1.5	0.6	1.5	0.9
8	0.0	2.1	2.6	1.6	1.3	—
Average	0.6	1.6	1.1	1.4	2.5	6

n agreement with the results of Gochberg & Reid (1966) and Wagner (1966) obtained by dilution techniques.

COMMENT

During the initial 3 hours following the intra-amniotic injection of hypertonic saline, a 30% increase in uterine volume has been calculated on the basis of both dilution and X ray techniques. This average 30% increase in uterine volume during the initial hours following hypertonic saline injections accounts for the early phase of "rapid acceleration" in the evolution of uterine activity (Pulkkinen & Kivikoski, 1969). Since the extent of the volume change appears to be variable from one patient to the next, variations in the early evolution of uterine activity can also be accounted for.

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TREATMENT OF CARCINOMA *IN SITU* OF THE CERVIX UTERI BY CONIZATION

A Five-year Follow-up

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Abstract. 226 patients were subjected to conization (including all cases with positive vaginal cytology and without clinical suspicion of invasion of the uterine cervix) at the Department of Gynaecology and Obstetrics, Malmö General Hospital, in 1963 and 1964. The operative technique was standard throughout the series which was followed up for 5 years.

Of the patients subjected to conization alone, nearly 95% showed no signs of recurrence at the end of the 5-year period. Late sequelae were few and mild. Fertility after the operation was high and the course of pregnancies normal.

Conization therefore is very valuable not only as a diagnostic method but also as an effective treatment for preinvasive carcinoma of the uterine cervix.

Opinions still differ on the choice of treatment for carcinoma *in situ* of the uterine cervix (1). Fear of undertreatment with consequent invasion of the cervix has induced many gynaecologists to adopt a radical attitude to the treatment of the condition. If vaginal smears or colposcopic findings are positive, punch biopsy is done (2–5). If histological examination of such specimens reveals cancer *in situ*, the patient is subjected to total hysterectomy (6–7), definitive treatment, or to conization, followed by hysterectomy (8).

Conization has been practised more widely since the advent of diagnostic vaginal cytology. So far conization has been used by most gynaecologists mainly for diagnostic (9–11) rather than for therapeutic purposes (12–19). Conization is considered superior to punch biopsy (20) for demonstrating and charting the extent of carcinoma *in situ* of both the portio and the cervical canal and for ruling out early invasion. Hysterectomy, on the other hand, is considered therapeutically superior to conization because it often reveals residual carcinoma *in situ* after previous conization (9, 21–26).

More or less radical total hysterectomy because of carcinoma *in situ* of the uterine cervix carries a greater risk of complications than conization, and may even prove fatal (6, 7, 19, 27). Even after total hysterectomy the patient must be followed up with cytological examination because of the risk of recurrence in the vaginal vault. Moreover, hysterectomy has a very important drawback, *viz.* sterility, which may create very serious problems for women below 40 years. The conservative procedure of conization seldom produces any morbidity and can also maintain fertility. It has, however, been stated that the frequency of abortion (12, 28) and premature delivery (29) as well as late sequelae (30) are increased after conization. The diagnostic advantages of conization are obvious, but its therapeutic effect has been questioned (11). In order to compare the conservative and radical treatments for carcinoma *in situ* of the uterine cervix we followed up all patients subjected to primary conization, 1963–1964, at the Department of Obstetrics and Gynaecology, Malmö General Hospital. We studied the 5-year cure rate of the carcinoma, the incidence of late sequelae, changes in fertility and the outcome of resulting pregnancies. 1963 and 1964 were the first 2 years in which all patients were treated by conization using a uniform surgical method devised in this department. Hysterectomy is now never per-

Table I *Primary conizations at the Department of Obstetrics and Gynaecology Malmö General Hospital 1963 and 1964*

Histopathological diagnosis		No.	Age (years) < 20	20–29	30–40	> 40
Clinically occult early invasive carcinoma	I A	5	—	—	—	5
Microcarcinoma	I A	5	—	1	1	3
Carcinoma *in situ*		147	1	49	51	46
Dysplasia		45	3	20	11	11
Normal appearance		15	1	4	3	7
Negative smear Erosion		9	—	5		2
Total		226				

formed before routine conization. If invasion is suspected clinically however further treatment is prescribed after a confirmatory biopsy Persistent or recurrent positive cytological smears during follow-up are regarded as evidence of insufficient removal of the intra-epithelial carcinoma and, as a rule as an indication for repeat conization or if the patient is over 40 and does not wish to have more children or has some co-existing uterine disease (such as myoma) often as an indication for hysterectomy

MATERIAL AND METHODS

111 patients were treated by conization in 1963 and 115 in 1964. Their progress during the 5 years until 1968–69 was studied from our hospital records, from information obtained from other hospitals where the patient had been seen in the meantime and from personal interview. The operative specimens were re-examined histologically with special reference to whether removal of the abnormal epithelium had been complete

The surgical technique was as follows:

On each side of the cervix No. chromium-catgut sutures are tied around the descending cervical branches of the uterine vessels. 40 ml of 0.5% Octapressin solution (Sandoz) is then instilled into the cervix to cause contraction of the vessels and arrest bleeding (32). Schiller's test is afterwards performed to assess the iodine-negative area of the portio as a guide for conization, which should be extended into dark-stained zones. The direction of the cervical canal and the distance between the internal and the external os is determined with a Hegar dilator The upper border of the cone should lie immediately below the internal os. The actual conization is then performed as cold-knife procedure, i.e. with the aid of a special knife *ad modum* Hillestrand; the cone is excised as a single piece with the portio as the base. The uterine cavity is curetted. If the cervix bleeds after conization, it is infiltrated again with Octapressin. The cervical wound is not sutured. The wound heals both anatomically and functionally (32). During the operation 200 ml of 5.5 glucose solution with the addition of 0.1 g epsilon-aminocaproic acid (0.4 g/ml) (Kabi) per kg bodyweight is given by intravenous drip.

This inhibitor of fibrinolysis is given to counteract post-operative bleeding (33). After operation the patient is given a further 6 g of epsilon-aminocaproic acid four times a day for 12 days orally for the same purpose, as well as an iron preparation to prevent haemorrhagic anaemia. As a rule, the patient may be sent home after 4–5 days.

Table II. *Survival and vaginal smear 5 years after conization*

	N	Death from intracurrent disease	Living symptom-free neg. VS
Carcinoma of the uterine cervix I A	10	—	9[a]
Carcinoma *in situ*	147	2[b]	145[c]
Dysplasia	45	2[d]	43

[a] Died in 1966 from an invasive carcinoma of the uterine cervix (stage IV)
[b] Breast cancer with metastases uraemia (chronic glomerulo-nephritis).
[c] 3 patients with dyskaryosis in their last vaginal smear
[d] Pancreatic carcinoma hepatitis

RESULTS

Of the 226 patients subjected to conization in 1963 and 1964 (Table I) the operation was carried out in 9 not for positive vaginal cytology but for an obstinate erosion. In 15 cases the surgical specimen was of normal histological appearance in spite of the fact that the vaginal smears had been regarded as positive Such false positive cytological diagnosis are most common (in about 1–) in elderly women Histological examination showed invasive carcinoma in 10 cones (in about 5 %) and carcinoma *in situ* in 147 In the remaining 45 there was suspect carcinoma *in situ* or only epithelial dysplasia.

Four of the women died within 5 years from intercurrent disease (Table II) It is noteworthy that the cause of death in – of these patients was extra-uterine carcinoma (pancreas, breast).

A ten-minute film of the operation (colour 16 mm, magnetic sound) is obtainable on request.

Table III. *Carcinoma in situ. Cone radical or not*

Figures in parentheses denote further operations because of later "recurrence"

Cone	No.	Age (years)			
		<20	20–29	30–40	>40
Radical	84	—	33	29 (1)	22 (1)
Not radical in endocervix	20	—	2	6	12 (4)
Not radical on portio	36	1	14 (1)	12 (6)	9 (3)
Not radical on portio or in endocervix	7	—	—	3 (1)	4 (2)
Total	147 (19)	1	49 (1)	50 (8)	47 (10)

A fifth patient in whom examination of the excised cone revealed invasion and who was subsequently treated by radiotherapy died 2 years later from advanced carcinoma of the uterine cervix. The remaining 197 patients were still alive after 5 years with no cytological signs of carcinoma (in 3 of them, however the last smear had shown atypical cells).

Judging from the histological findings the operation had been radical in 84 (57%) of the patients with carcinoma *in situ* in the excised cone (Table III). But in the remaining 63 the line of excision had passed through tissue with carcinoma *in situ*. It was mainly in elderly women that the operation had not been sufficiently radical. Yet 46 of these 63 patients have become symptom-free and showed no subsequent cytological evidence of a recurrence or residual carcinoma. Further treatment had been necessary in only of the cases in which the primary operation had been judged as radical and in 17 of those in which the primary operation had not been considered radical.

The additional treatment in these 19 cases with recurrent positive cytology aftert primary conization consisted of repeat conization in 9 hysterec

T ble IV *Recurrence of carcinoma in situ after conization and its treatment*

Primary cone	No.	"Recurrence	Time for first positive vaginal smear	Treatment of recurrences
Radical	84	2	4–8 months after conization	1 repeat conization 1 hysterectomy
Not radical in endocervix	20	4	2–8 months after conization	4 hysterectomy
Not radical on portio	36	10	2 24 months after conization	6 repeat conization 4 hysterectomy
Not radical on portio or in endocervix	7	3	3–8 months after conization	2 repeat conization 1 radiotherapy
Total	147	19		9 repeat conization 9 hysterectomy 1 radiotherapy

Table V *Results of conization in cases f dysplasia*

	No	Later appearance of carcinoma in situ		
		No	First positive smear after conization	Treatment of the "recurrences
Cone radical	38	3	2–24 months	2 repeat conization 1 hysterectomy
Cone not radical	7	2	2–9 months	1 repeat conization 1 hysterectomy
Total	45	5		3 repeat conization 2 hysterectomy

Table VI *Sequelae 5 years after conization*

Dysmenorrhoea	7[a]
Dyspareunia	2
Menorrhagia	12
Secondary sterility	2
Total number of women examined	194

[a] Including 2 patients *who had been subjected to a second* cone biopsy in the meantime.

tomy in 9 and radiotherapy in 1 patient (Table IV) Additional indications for hysterectomy were myoma in 2 cases, a request for legal abortion with sterilization in one case, and in 5 cases the women were over 40 years of age. In the case treated by radiotherapy repeat conization was avoided because the patient was mentally deficient. Repeat conization could have been considered in these cases.

Of the 45 cases where only moderate dysplasia had been found in the primary cone despite positive vaginal cytology later cytology had been repeatedly positive in 5 (Table V) and a further operation (conization) had revealed carcinoma *in situ* histologically All of these patients are still alive and symptom-free and have shown no cytological signs of recurrence

Late sequelae after conization were relatively rare (Table VI) and consisted mainly of dysmenorrhoea in 3–4% and menorrhagia in 6% The dysmenorrhoea, which was always mild, was probably due to some degree of stenosis of the cervical canal with consequent obstruction of the menstrual flow It is noteworthy that ? of the 7 patients with dysmenorrhoea developed this symptom after a second conization. One of the ? women with secondary sterility (Table VI) had failed to conceive after 1 1/2 years of normal marital life with a new husband, and the other had *remarried a man with a high percentage of abnormal forms of spermatozoa in his semen.*

On the other hand fertility after conization was high in the present series (Table VII). There have been altogether 70 pregnancies. Of the 44 full-term infants, 4 had been born after repeat conization. Of the premature infants, 3 weighed *1 500* grams, *2 320 g and 2 400 g, respectively* but all developed normally Two premature infants weighed 860 g and 2 450 g, respectively but 1 year later each of the mothers gave birth to a child weighing 3 340 g and 3 630 g, respectively without having received any treatment in the meantime. One premature child was born to a woman who had failed to conceive during 5 years normal marital life before the operation (husband had oligospermia). One year after conization this patient aborted spontaneously in the 6th month, but afterwards has given birth to children weighing 2 950 and 2 300 g. Just before the conization one woman had a premature child weighing 1 210 grams, but after conization she again conceived and was delivered of a normal child at term. No cervical cerclage has been used in our patients.

Table VII *Fertility after conization in women under 40 years*

Contraception	67
Primary sterility	9
Secondary sterility	2
Conceived	55
Total number under 40 years	133
Outcome of pregnancies	
Induced abortions	11[a]
Spontaneous abortions	6[b]
Premature deliveries	6
Term deliveries	44[c]
Currently pregnant	3
Total number of pregnancies	70

[a] 1 after repeat conization.
[b] 5 in 1st trimester 1 in 2nd trimester (or 6th month).
[c] 4 after repeat conization.

DISCUSSION

Many gynaecologists consider conization inadequate as definitive treatment for carcinoma of the uterine cervix stage 0 (carcinoma *in situ*) because later hysterectomy has often revealed residual *in situ* changes (9 21–27) Conization has therefore often been used only as a diagnostic method. But if it could be proved that conization also has a permanent therapeutic effect in a high percentage of cases, it would not only avoid unnecessary hysterectomy but also preserve fertility

Of the patients subjected to conization in 1963 and 1964 at the Department of Gynaecology and Obstetrics, Malmö General Hospital, because of carcinoma *in situ* the operation, as judged from histological examination of the excised cones,

was not radical in about 40%. Nevertheless, 73% of these women were afterwards symptom-free and cytological smears became negative without any further treatment (Table III). This thus shows a tendency to spontaneous healing after conization and suggests that conservative surgical treatment is adequate.

It is difficult to say whether the *in situ* changes found at a second conization should be regarded as true recurrences or as residual changes (Tables IV and V). That they were probably residual lesions is strengthened by the observation that in nearly all these patients vaginal smears became positive again within 1 year of primary conization. This also shows how necessary it is to follow up these patients cytologically at close intervals especially during the first year after conization. Careful cytological follow-up is, of course, always necessary after conservative treatment of carcinoma *in situ*.

Of the patients subjected solely to conization, no signs of carcinoma were later demonstrable in almost 95%. The remaining 5% who were subjected to hysterectomy or (in one case) to radiotherapy could possibly also have been treated by repeat conization. Conization is thus very valuable in the treatment of carcinoma *in situ* of the uterine cervix.

Almost all of the patients who wished to have a child became pregnant (Table VII). In some cases pregnancy had evidently occurred unintentionally and remarkably quickly resulting in a high frequency of legal abortions. It is possible that conization removes a sterility factor and that the high fertility after the operation often surprised the women.

The courses of the pregnancies were normal. Thus, there was no suggestion that the frequency of spontaneous abortion or premature birth was abnormally high. Neither did the investigation produce any evidence that the operation had caused cervical incompetence. Therefore cervical cerclage in these cases should be avoided.

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RESPONSE OF THE PREGNANT HUMAN UTERUS TO LOW AND HIGH DOSES OF PROSTAGLANDIN E_1 AND E_2

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Abstract. Uterine sensitivity to low doses of prostaglandin E_2 was compared at midpregnancy and at term. An analysis of the amniotic pressure recordings revealed that the uterus in midpregnancy responded by increased contractility to an infusion rate of the same order as that, commonly used for induction of labour at term. However the absolute intensity of the contractions was significantly higher at term than at midpregnancy. Intravenous infusion of high doses of prostaglandin E_1 (maximum dose without consistent subjective side effects) at midpregnancy stimulated the uterus to frequent contractions (6–8 contractions per 10 min) of gradually increasing intensity which reached an average value of 35 mmHg following 6 hours of infusion. The individual uterine response to prostaglandin varied considerably from one case to another. The "maximum tolerable infusion rate" which could be administered without major subjective side effects also varied within a wide range.

Experimental and clinical evidence indicates, that the sensitivity of the human uterus to oxytocin is markedly increased during the course of pregnancy (2, 16, 18, 26). Thus, it is more or less impossible to achieve an expulsion of the conceptus during early pregnancy by administration of oxytocin (7), whereas comparatively low doses may induce effective uterine contractions at term (16, 18 28, 41 42, 44 45).

In contrast the prostaglandins may stimulate effective uterine contractions also during early pregnancy as evidenced by the fact that therapeutic abortion may be accomplished by intravenous infusion of prostaglandin E_1 (PGE_1), E_2 (PGE_2) and $F_{2\alpha}$ ($PGF_{2\alpha}$) (13, 22, 24 29 30 31 39 46, 47 48). It has been claimed, that the sensitivity of the pregnant human uterus to these substances is virtually independent of gestational stage (10, 11 22, 39). However this statement has not been supported by adequate quantitative studies, nor has any quantitative analysis been performed on the character and magnitude of uterine activity following high doses of the same order as those, used for induction of abortion.

The present study was made to investigate the response of the human uterus in midpregnancy to continuous intravenous infusion of low and high doses of prostaglandin. The low infusion rate corresponded to the one, used for induction of labour at term, and the high dose level corresponded to the maximum tolerable dose.

MATERIAL AND METHODS

The case material included 16 women in the 16th to 18th week of pregnancy admitted to the hospital for therapeutic abortion and 8 pregnant patients at term (37th to 42nd week of pregnancy), in whom labour was induced, on account of Rh immunization or postmaturity. For the purpose of comparison only pregnant women at term, in whom the membranes remained intact during the prostaglandin stimulation, were included in the latter series.

Prostaglandin E_2 was administered intravenously at constant rate of 0.7 µg per minute to 8 women in midpregnancy and 8 at term for at least 6 to 7 hours. Another group of 8 patients in midpregnancy received prostaglandin in stepwise increased doses, until maximum dose without consistent subjective side effects ("maximum tolerable infusion rate") was attained. Since PGE_2 was not available, the latter group of patients received PGE_1. However, earlier results indicate, that these two prostaglandins have approximately the same qualitative and quantitative effect on uterine contractility (8, 9 11 12, 49). In most cases the tolerance dose of PGE_1 varied between 2.5 and 5.0 µg per minute, although the dose range was as wide as 1.5 to 10.0 µg per minute in individual instances.

Uterine contractility was recorded by measuring the amniotic pressure according to the method of Caldeyro-Barcia et al. (14). The tracings from all cases were analysed by measuring the intensity of the contractions in mmHg and their frequency per 10 min. From these

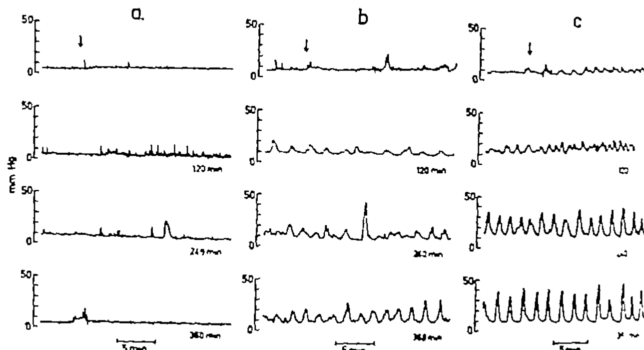


Fig 1 Individual differences in uterine response to prostaglandin (PGE_2) Intravenous infusion of PGE_2 at a constant rate of 0.7 μg per minute to three women in midpregnancy The tracings illustrate contractility at the beginning of the infusion (upper curves, the arrows indicate initiation of infusion) and following 120, 240 and 360 min of stimulation. (*a*) Para 3, gravida 5 31 years, 18th week: no obvious influence on uterine motility during the infusion period. (*b*) Gravida 1 19 years, 19th week: development of small irregular contractions. (*c*) Para 1 gravida 4, 23 years, 18th week: contractions of gradually increasing intensity and coordination.

values the uterine activity in Montevideo Units (M.U.) was calculated. Only a distinct elevation of the uterine pressure exceeding 4 mmHg was identified as a contraction. Mean values of intensity and frequency were calculated from each tracing during 30 min before the infusion and for every 30 min period throughout the experiment. Resting tone level was measured at the end of every 10 min period. Vaginal examination was made before and after the infusion and in the patients at term also during the course of the infusion.

RESULTS

Low Dose Stimulation (0.7 μg PGE_2 per minute)

Midpregnancy

The individual uterine reaction to the same dose of PGE_2 varied considerably Different types of response are illustrated in Fig. 1

In two cases there was no significant increment of uterine motility (Fig. 1 *a*)

Four of the tracings showed a stimulatory response in terms of small irregular contractions (Fig. 1 *b*)

In the remaining two cases there was an increasing motility at first irregular thereafter on a more coordinated basis with a comparatively high intensity of the contractions (Fig. 1 *c*)

Uterine tonus was only slightly influenced by the infusion. However an elevation above the pre-infusion level occurred in all cases and varied generally between 2 and 5 mmHg. The highest tone elevation observed was 11 mmHg.

No bleeding or signs of cervical effacement or dilatation could be noted at the end of the infusion period in any of the cases. After termination of the prostaglandin infusion all patients were given an intraamniotic injection of hypertonic saline. The development of the saline induced abortions in these cases did not differ from the generally observed course. All patients completed their abortions within a mean period of 33 hours ±6 following the injection.

Term pregnancy

Fig. 2 illustrates a typical tracing from one of the subjects at term. At this stage of gestation there is a certain degree of spontaneous uterine activity In the majority of the cases this activity appeared as contractions of low intensity and frequency which under our experimental conditions corresponded to a uterine activity of 37 M U ±22

During the prostaglandin infusion there was an increase in the intensity and frequency of the contractions, although this response was very slight in two of the subjects. The contractions were mainly regular and labour like, but short episodes of incoordination occurred. Resting tone level was not influenced by the infusion except for transient periods of incoordination.

The cervix was not effaced and cervical dilatation did not exceed 1–2 cm at the beginning of the infusion. Following 360 minutes of prostaglandin stimulation 50–75% effacement of the cervix was evident in three of the subjects, but there was no sign of progressing cervical dilatation in any of the cases.

High Dose Stimulation (1.5–10.0 μg PGE₁ per minute)

High doses of PGE_1 and PGE_2 given intravenously are associated with increased heart rate and subjective side effects such as nausea, headache and dysmenorrhoic pain (4 5 11 19 20, 49). For obvious reasons high doses of a uterine stimulant cannot be administered to a woman in late pregnancy with living foetus.

Fig. 3 illustrates the development of uterine contractility in two women in midpregnancy given PGE_1 at a maximum tolerable infusion rate. One of the patients (Fig. 3) obtained nausea at a dose of 2.5 μg per minute, for which reason the rate had to be reduced to 1.5 μg per minute. The second patient (Fig. 3 *b*) tolerated 9.0 μg per minute without any subjective side effects. After six hours of infusion she started to complain of slight nausea. These two cases illustrate the individual difference in subjective tolerance to high doses of PGE_1. It should be noted, that the development and magnitude of uterine activity was largely the same although the prostaglandin dose differed markedly.

The initial effect on uterine contractility was characterized by an elevation of tonus, which started 2 to 5 minutes following the beginning of infusion. A state of uterine contracture developed with a maximum tone level of 15 to 35 mmHg. Approximately 30 min later there was a gradual decrease of tonus and at the same time an increase in the intensity of the contractions. Uterine activity increased gradually and reached a magnitude which, if sustained, is usually sufficient for expulsion of the conceptus. At vaginal examination following 360 min of stimulation it was found, however that the cervix was uneffaced and closed except in one subject, where the foetus had been expelled into the dilated cervix.

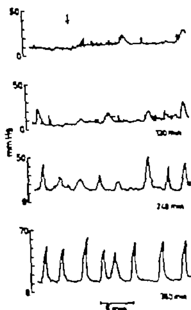


Fig. 4. Influence of intravenous infusion of PGE_1 at constant rate of 0.7 μg per minute on uterine contractility of pregnant woman at term para 4 gravida 5 31 years, 38th week. Spontaneous uterine activity as low (upper curve, the infusion initiated at arrow). Note the gradual development of labour like activity

Comparative Analysis of the Mean Curves

A comparison of the uterine response to low doses of prostaglandin at midpregnancy and at term as well as to high doses at midpregnancy is illustrated by the diagrams in Figs. 4 and 5

The results indicate that the in midpregnancy uterus does react by increased contractility to the comparatively low doses of prostaglandin that are commonly used for induction of labour at term (3, 23 29 32, 33, 34 38). A stimulatory response was obtained in 6 out of 8 subjects. Following the initial response there was further increase of the intensity of the contractions during the course of the infusions. Intensity calculated from the individual tracings at 360 min was significantly higher ($P < 0.05$–0.001 Student's *t*-test) than the corresponding values at 60 min. The mean values obtained in 4 of the curves correspond approxi-

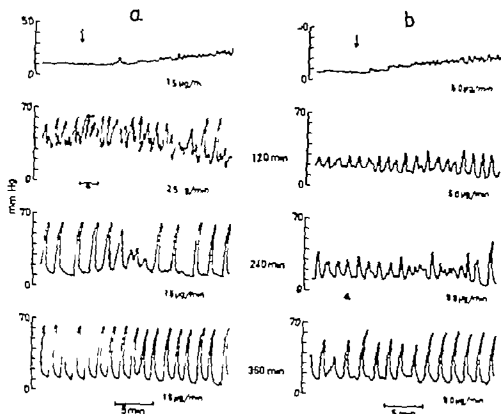


Fig 3 Influence on uterine contractility of intravenous infusion of prostaglandin (PGE$_1$) at maximum tolerable doses (midpregnancy). The tracings illustrate uterine contractility at the beginning of the infusion (upper curves, the arrows indicate initiation of infusion) and following 120 240 and 360 min of stimulation. (a) Gravida 1 22 years, 16th week: a dose of 2.5 µg per minute was accompanied by episodes of nausea and vomiting (*) and the infusion rate was reduced to 1.5 µg per minute. (*b*) Gravida 1 21 years, 16th week a dose of 5.0 µg per minute was given without any side effects and at (A) the infusion rate was increased to 9.0 µg per minute. The dose was well tolerated and maintained for the remaining observation period. Note the similarity of the tracings in spite of the great difference in infusion rates.

mately to the mean curve in Fig. 4 The large S.D reflects the fact, that in two cases the intensity reached a level of 20–30 mmHg whereas no measurable uterine activity was found in two of the tracings.

The mean values representing frequency are greatly influenced by the two cases without any uterine activity In the remaining 6 subjects the individual values following the first hours of infusion stabilized within a range of 4–7 contractions per 10 min. Mean uterine activity reached a level of 50–60 M U in this group.

Mean intensity was significantly higher ($P < 0.01$ Student's *t*-test) at term than at midpregnancy during the entire infusion period. The individual values varied considerably also in this group, but in 5 of the 8 cases there was a significant increment ($P < 0.05$–0.001 Student's *t* test) of the intensity during the course of the infusion.

Frequency increased in all cases except one, and it should be noted that in 4 out of 8 subjects frequency exceeded 5 contractions per 10 min, which is not acceptable from a clinical point of view (17). Uterine activity corresponded to 140 M U at the end of the infusion period in the group.

High doses of PGE$_1$ administered at midpregnancy caused a very rapid increment of frequency in all cases during the first hour and then a stable level corresponding to 6–8 contractions per 10 min. In contrast there was a continuous augmentation of the intensity during the infusion, and at the end of this period the mean values were comparable to those seen at term. Due to the high frequency at midpregnancy uterine activity reached a level of 250 M U

DISCUSSION

It is quite evident that less "uterine work" is required to complete delivery near term than to expel the conceptus in midpregnancy since resistance

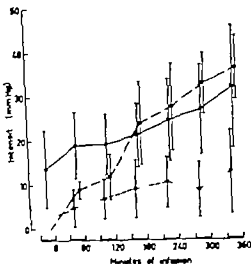


Fig. 4. Graphic illustration of the development of uterine contractility (intensity of the contractions) during intravenous infusion of prostaglandin. High dose stimulation corresponded to maximum tolerable dose and varied between 1.5 and 10.0 μg per minute. Each of the three curves represents mean values (± S.D.) from 8 tracings. The individual values correspond to the average intensity during every second 30 min period of the tracing. —— At term PGE, 0.7 μg/min, Midpregnancy PGE, 0.7 μg/min, — — Midpregnancy PGE, high dose.

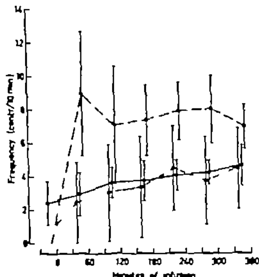


Fig. 5. Development of frequency of the contractions. Consult the text of Fig. 4. —— At term PGE, 0.7 μg/min, Midpregnancy PGE, 0.7 μg/min, — — Midpregnancy PGE, high dose.

to expulsive forces is related to the degree of cervical ripeness (1, 6). It is, therefore, impossible to evaluate uterine sensitivity to stimulating drugs on the basis of the clinical outcome of a time limited induction trial. Uterine sensitivity must be evaluated by a quantitative analysis of uterine contractility.

Earlier results show that administration of a certain prostaglandin dose induces a progressive increment of uterine activity, and that a considerable time has to pass, before stable activity is present (38). Therefore, the same infusion rate of prostaglandin was maintained for an observation period of 6 hours. However, the activity of the pregnant uterus increased continuously throughout the infusion period in the majority of the cases and never reached a state of stable contractility. This tendency to an accumulative response is in some contrast to the corresponding pharmacological effect of oxytocin (40).

The problem, whether the uterus at term is in fact more sensitive than in midpregnancy has to be assessed from the development of the curves. Spontaneous contractility differed significantly in midpregnancy and at term. If the uterine response is compared in terms of the *increment* of intensity above the level of spontaneous contractility, then no significant difference can be found ($P > 0.2$, Student's t-test). However, judged from the absolute mean values the fact remains, that the uterus at term developed nearly three times higher intensity values than the smaller uterus (32 mmHg ±14 and 13 mmHg ±9 respectively). The significant difference between these pressures ($P < 0.01$, Student's t-test) is particularly striking, if the small radius of the uterus in midpregnancy and its comparatively large wall thickness is taken into consideration (27, 43). These last mentioned factors would favour high pressures in midpregnancy. Since the results indicated the contrary, it seems reasonable to assume, that the same dose of prostaglandin induced higher tension in the myometrium at term than in midpregnancy. However, by making this conclusion it should be remembered, that the contraction complexes developed by the uterus in midpregnancy indicated uterine incoordination in many instances. This means, that probably only a segment of the uterine wall was active, whereas the surrounding segments might have been

stretched which invalidates the effectiveness of the contractions (21)

The patients at term included in the present study were selected from a larger induction series, where rupture of the membranes and cervical dilatation in some cases occurred during the course of the 6 hour stimulation period. Rupture of the membranes and/or commencement of active labour may enhance uterine activity by intrinsic mechanisms (18 35) The case material was selected to avoid such interference, although it is appreciated that the contractility curves thus may represent those patients with minimum sensitivity

Infusion of 0.7 μg PGE_2 per minute for 6 hours stimulated the uterus at term to a mean activity of 140 M U and in midpregnancy to 50–60 M U If the same levels of activity should be reached by infusion of oxytocin, the approximate doses are 4–8 mU per minute (at term) and 16–32 mU per minute (16th–18th week) (2, 16, 18) This means a difference in infusion rate of 1 4 which should be compared with 1 1 for prostaglandin. In other words, the difference in sensitivity to oxytocin at term and at this stage of midpregnancy is probably larger than the corresponding difference in sensitivity to prostaglandin.

If the infusion rate of prostaglandin (PGE_1) was increased approximately 5-fold, a much higher level of activity was obtained. The intensity values reached a mean of 36 mmHg and the contractions tended to aquire better coordination. It has been shown that these pressures are sufficient for inducing cervical dilatation at least at term (15 36) and that following a significant period of stimulation, such doses may result in an expulsion of the conceptus in midpregnancy (24 31) Uterine activity following 6 hours of stimulation with a comparatively high dose of PGE_1 was around 250 M U This high activity is, however much lower than the one obtained by stimulating the uterus in early pregnancy (7th–8th week) with equivalent doses of $PGF_{2\alpha}$ (13 48). The difference in pressures is again probably due to the difference in uterine radius and is also reflected in the high success rate of induction of abortion in early pregnant women (13 46, 47 48)

An interesting observation with bearing on the clinical use of prostaglandins was the relation between subjective side effects and the magnitude of the uterine response. Patients who only tolerated e.g. 1.5 μg PGE_1 per minute developed uterine activity of approximately the same order as those tolerating 10.0 μg per minute. This may, at least in part, be due to the efficiency of inactivating enzyme systems. If inactivation is rapid, the resulting plasma concentration of PGE_1 may be low and permit high doses, before subjective side effects appear If on the other hand, inactivation is slow only low infusion rates can be administered and uterine activity will still be high, due to a comparatively high plasma concentration (25 37 50 51 52). The same phenomenon may also be reflected by the great difference in the individual uterine reaction to the same infusion rate of PGE_2 both in midpregnancy and at term. This difference reduces the possibility of recommending standard doses for clinical use and creates a considerable difficulty in calculating an average level of reaction from a small series.

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INFLUENCE OF ETHANOL ON THE RATE OF GALACTOSE ELIMINATION IN WOMEN TAKING ORAL CONTRACEPTIVES AND IN PREGNANT WOMEN

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Abstract Peroral galactose tolerance tests with and without ethanol intake were performed on pregnant women with various clinical diagnoses, on women taking oral contraceptives and on control patients. The influence of ethanol on the rate of galactose elimination was normal in the control group and the group taking oral contraceptives. In half of the gravidae a diminished effect of ethanol on galactose elimination was observed. This indicates that in many pregnant women ethanol does not reduce the redox state of the liver.

Normally in rat and human livers ethanol results in a negative shift of the redox state of the liver (1 3 10 16). In fatty liver induced by a choline deficient diet, the capacity of the liver to form or use extramitochondrial reducing equivalents is changed in such way that no shift in the redox state of the liver can be observed during ethanol oxidation (14). Measurements of the redox potential of the rat liver using the galactose tolerance test have demonstrated that the main reason why ethanol no longer causes this shift is, in some as yet unexplained way related to a lack of choline (11 15). In normal human subjects the elimination of galactose is strongly inhibited by an ethanol load (12). This inhibition does not occur in human alcoholics whose food consumption has long been inadequate (12). Recently it has been found that this failure of inhibition is probably caused by protein deficiency (13), and on this basis the galactose tolerance test with ethanol can be used for the early diagnosis of protein-deficient fatty liver.

Since no information concerning the galactose tolerance test with ethanol in pregnant women and women using oral contraceptives is available, a comparative study was made between these groups of women. Peroral galactose tolerance tests with and without ethanol loading were performed on these subjects and on control women.

METHODS

The galactose test was begun in the morning, the patient having fasted the previous night. The patient was asked to drink 10% non-sterile galactose solution as rapidly as possible, the total amount of galactose given being 350 mg/kg body weight. Blood samples were taken from finger tip at 40, 60 and 80 min. This version of the galactose tolerance test is called Peroral Galactose I in this paper. In the Peroral Galactose II test, 300 mg of ethanol per kg body weight was given as 15% solution by mouth 15 min before galactose loading; otherwise the test was the same as Peroral Galactose I. The galactose concentration of the blood samples was determined enzymatically by the method of de Verdier & Hjelm (20) and Hjelm (5), using the galactose oxidase reagent produced by AB Kabi (Stockholm, Sweden). The values were plotted against time on millimetre paper, the differences between the galactose serum levels (Peroral Galactose II minus Peroral Galactose I) at 40, 60 and 80 min were determined (A, B, C, Fig. 1), and the differences summed. In peroral galactose tolerance tests the sum of the differences (A + B + C, Fig. 1) reveals the amount of inhibition. This test has previously been compared with an intravenous galactose test and the results of the two tests found to be comparable. The study will be published in detail later (7).

MATERIAL

Normal values for Peroral Galactose I and II were determined in 18 women of child-bearing age. None of these subjects were hospital patients and none of them had any clinical signs of disease, nor were they taking

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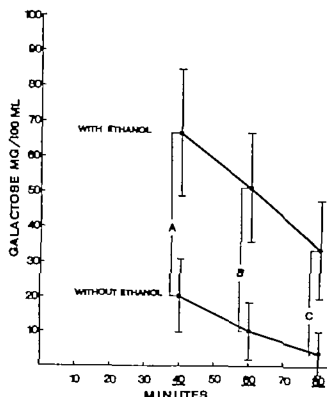


Fig 1 Time course of the plasma concentration of galactose after galactose ingestion in control subjects and the influence pon it of ethanol loading. Each point represents the mean value of eighteen experiments and the bars represent standard deviation. *A B* and *C* The differences of the serum galactose levels (Peroral Galactose II, with ethanol, minus Peroral Galactose I, without ethanol, at 40, 60 and 80 min.)

any drugs, including oral contraceptives. Their mean age was 22 years, with a range of 20 to 32 years.

The subjects taking oral contraceptives had been using various preparations, including norgestrel + thinyloestradiol (Primovlar®), norethisterone + mestranol (Ortho-Novin® Ortho-Novin mite®), megestrol acetate + mestranol (Delpregnin®), norethisterone acetate + ethinyloestradiol (Anovlar®), chlormadinone acetate + mestranol (Aconcen®) and lynestrenol + mestranol (Ovanon®) They were taking no other drugs and had no disease. They had never suffered from any side-effects from the use of ral contraceptives except oedema, increase in weight or occasional headache. The mean duration of taking oral contraceptives was 15 months, with range f 1 to 7 months. The day f the menstrual cycle on which the test was performed in these subjects was distributed evenly through the beginning, middle and end of the cycle. Fourteen women were examined. Their mean age was 27 years, with a range of 19 to 46 years

The gravidae were women admitted to hospital for examination o therapy because of toxaemia, hypertension, cervical erosion, anaemia, threatened abortion or uterine contractions (Table I). There was no clinical suspicion of hepatic disease. Depending on the clinical diagnosis, they were receiving antibiotics, vitamins, antihypertensive drugs, spasmolytics or alcohol (some cases f threatened abortion). Twenty-seven pregnant subjects were examined (Table I), their mean age being 22 years, with a range of 19 to 34 years. The mean duration of pregnancy was 27 weeks, with a range of 8 to 40 weeks.

RESULTS

Figure 1 shows the serum galactose values at 40, 60 and 80 min with and without ethanol loading in the 18 control subjects. The serum galactose values with ethanol loading are essentially higher than without ethanol loading, indicating the inhibition of galactose elimination caused by ethanol.

The results in Table II reveal the sums of the differences in serum galactose levels with and without ethanol loading in controls, pregnant women and women taking oral contraceptives. The results in the group using oral contraceptives

Table I The parity duration of pregnancy and complications of pregnancy together with the sums of the differences in serum galactose levels (Peroral Galactose II minus Peroral Galactose I at 40 60 and 80 min A + B + C Fig 1) among 27 pregnant women

Duration of pregnancy (weeks)	Parity	Complication of pregnancy	Sum of the differences (mg/100 ml)
8	II	Threatened abortion	71
11	I	Threatened abortion	72
11	III	Diabetes mellitus Hypertension	45
13	III	Threatened abortion	174
18	VIII	Deep thrombosis of fem. vein	84
19	I	Threatened abortion	73
20	II	Cervical incompetence	71
20	III	Uterine contractions	66
21	V	Threatened abortion	63
22	I	Anaemia	82
23	II	Indefinite bdominal pains	9
29	I	Cervical erosion	89
30	I	Hypertension	98
30	I	Hypertension	73
30	I	Uterine contractions	74
30	I	Uterine contractions	95
33	I	Cervical erosion	0
33	III	Anaemia	80
33	I	Toxaemia	46
35	II	Uterine contractions	68
35	I	Hypertension	34
35	I	Ureteral stone	3
36	III	Uterine contractions	0
39	I	Toxaemia	22
39	I	Normal pregnancy	100
40	II	Toxaemia	54
40	I	T xaemia	80

were essentially the same as in the control subjects. In the pregnant subjects, the influence of ethanol on galactose breakdown was weaker than in either of the other two groups. Although the sum of the differences averaged only 64.0 mg/100 ml ± 37 (S.D.), about half the pregnant women seemed to have normal inhibition of galactose elimination after ethanol intake (Table I). Statistically these results in the group of pregnant subjects differ highly significantly from those of the control group (Table II). This lack of inhibition was not related to the parity clinical diagnosis or duration of pregnancy in this series.

Table II. *The sums of the differences in serum galactose levels (Peroral Galactose II minus Peroral Galactose I at 40 60 and 80 min = A + B + C Fig I) in controls, pregnant women and women using oral contraceptives*

The results are given as means ± standard deviation

Group	No. of patients	Sum of the differences mg/100 ml (A+B+C)	Significance
Controls	*18*	*115.9 ± 41 (S.D.)*	—
Women using oral contraceptives	14	115.7 ± 35 (S.D.)	—
Pregnant women	27	64 0 ± 37 (S.D.)	$p < 0.001$

DISCUSSION

Alcohol is oxidized to acetaldehyde and acetate by two enzymes, alcohol dehydrogenase and acetaldehyde dehydrogenase, and both these reactions lead to the transfer of hydrogen to NAD which is reduced to $NADH_2$ (8, 21). This induces a shift in the NADH/NAD ratio in more reduced direction, indicating that the redox state of the liver becomes more negative (4).

Fatty livers induced by a choline-deficient low-protein diet have been found to have a greater capacity to oxidize cytoplasmic NADH or to have a different mechanism for controlling the cytoplasmic redox state (14, 15).

One of the key reactions in the metabolism of galactose is the step involving the conversion of UDP-galactose to UDP-glucose. This reaction is catalyzed by the enzyme UDP-galactose-4-epimerase. Maxwell has shown (9) that the coenzyme in this epimeration is NAD and that $NADH_2$ is a potent inhibitor of the enzyme. As mentioned above, during ethanol oxidation large amounts of $NADH_2$ are formed. As result, in normal individuals the capacity of the liver to *oxidize galactose* is decreased (17 18, 19).

With the galactose tolerance test as a measure of the redox state of the liver it has been possible to demonstrate that ethanol does not increase the $NADH_2$/NAD ratio in human alcoholics whose food consumption has been inadequate for long periods (12). According to previous animal experiments, this may indicate some kind of metabolic alteration in liver function induced by choline deficiency (15). Subsequently it has been shown that treatment with a high-protein diet normalizes the galactose tolerance test with ethanol in 4 weeks in human subjects (12, 13).

In a pregnant woman whose liver exhibits diminished inhibition of galactose elimination after an ethanol load, there may be a deficiency of methionine, because the developing foetus might draw off a great deal of the methionine pool for its own synthesis, leading to deficient choline synthesis, with the result that a shift in the redox state of the liver would not be demonstrable. The situation would be about the same as in human alcoholics after inadequate food consumption. This hypothesis is favoured by the fact that the shift of the redox state does not occur in the livers of all pregnant women. Perhaps those in whom it does occur have an adequate amount of choline or methionine in their daily food.

On the other hand, ethanol is known to increase the reduction of 17-ketosteroids into their dihydroxy forms (2). This reaction will use $NADH_2$, and the greatly increased steroids produced during pregnancy could consume part of the NADH formed during ethanol oxidation. This might afford an alternative explanation of why ethanol loading does not induce the inhibition of galactose elimination in some pregnant subjects. However the results for the group taking oral contraceptives are essentially the same as those for the control group. Thus synthetic oestrogens combined with progestogens do not alter the redox state and the metabolism of galactose in the liver. However the quality and quantity of the steroids in oral contraceptives differ markedly from those produced during pregnancy and the results ob-

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tained for this group of subjects cannot be compared directly with those obtained in the pregnant subjects.

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PLACENTAL LOCALIZATION BY THE GAMMA CAMERA AND DOPPLER FLOWMETER

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Abstract. The placenta was localized in 99 women, between the 35th and 40th week of pregnancy by both the Doppler flowmeter and the gamma camera, using 300 μCi ^{99m}T as the tracer. The conclusion from each method was the same in 84 cases (85%) and different in 15 cases. In only 4 of these latter cases was the placenta located in opposite quadrants of the uterus by the two methods. According to the results obtained, use of the Doppler flowmeter is suitable screening test.

Knowledge of the precise position of the placenta is of great value in the management of ante partum haemorrhage and prior to amniocentesis. Radiological methods of placental localization are widely used and provide useful information in centres familiar with the technique and the interpretation of the films. Soft tissue placentography is, however, unreliable prior to 34 weeks of gestation and it is frequently necessary to localize the placenta earlie than this, particularly in cases of severe rhesus disease.

New reliable methods which do not involve the use of X-rays, ha been discovered in recent years. Isotope placentography using radioactive tracers and a γ-ray radiation counter is approximately 90–100% accurate. It has been found to be a safe method of investigation in which the total amount of radiation to the fetus is at the most 1 000 mrad μCi (Hibbard, 1961 Laakso et al., 1964). Anothe method under trial is based on an ultrasound technique. The Doppler flowmeter a simple pparatus based on detective changes in ultrasonic w es, is in use t many maternity hospitals. In addition, major hospitals use A and B-scan ultrasonic apparatus based on similar principles to help with obstetrical diagnosis (g. Kratchowill, 1966 Bishop 1956 Piiroinen & Manninen, 1970).

Our purpose in this study was to compare the results of placental localization by the gamma camera and the Doppler flowmeter based on the ultrasonic technique.

MATERIAL AND METHOD

The series consisted of 99 cases on hich both ultrasound and isotope studies were performed between the 35th and 40th week of pregnancy.

The surface of the uterus was divided into five zones (I–V) as shown by Table I and the position of the placenta as determined accordingly. The upper segment was divided into four quadrants. Zone V represented the lower segment.

A Doptone device (Smith Kline) as used in the Doppler method.

The surface of the uterus was examined at intervals of 3 cm with transducer.

The placenta gives typical humming sound which reminds es the don of factory hall.

A gamma camera (Nuclear Chicago PHo/Gamma III) as used in the isotope study. The patient was given 300 μCi of ^{99m}Tc-pertechnetate by intravenous injection and the pictures were taken immediately afterwards.

Before the isotope injection the patient was given potassium perchlorate solution for 3 days to block both the maternal and fetal thyroid gland.

RESULTS

The 84 (85%) cases in which the placenta was localized in the sam quadrant by both methods are presented in Table I. The majority of the placentas were in the fundus (57 cases). There were no differences in localization between normal pregnancy and cases with toxemia of pregnancy and no cases of placenta praevia.

Table II shows the cases in which the placenta was not located in the same quadrant by each

tained for this group of subjects cannot be compared directly with those obtained in the pregnant subjects.

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perience is required to perform the investigation accurately especially if the placenta is located on the posterior wall of the uterus. The reliability of the Doppler flowmeter according to our studies, is adequate as a screening test. If it leaves the position of the placenta in doubt, additional investigation by X ray or isotope placentography is indicated.

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Table I *The 84 cases in which the position of the placenta determined by the Doppler flowmeter and gamma camera was the same grouped schematically on the surface of the uterus*

Right Left

I II III IV V

	I	II	III	IV	V	
	12	3	8	3		6
×	18	21	10	5		54
o	1	2		1		4
	31	26	18	9		84

Normal pregnancy
× M ld p e eclamps a
Seve e pre eclamps a

method. However there were only 4 cases (nos. 7 41 48 and 61) in which the methods placed the placenta in opposite quadrants. In 11 of these cases the placenta was located in adjacent qua drants (either on the same side of the uterus or above or below the umbilicus)

DISCUSSION

The results obtained by Doppler flowmeter and by X-ray placentography were compared by Brown (1967) who established the error of the ultrasonic technique to be about 10% Accord ing to Jonson and his co-workers (1968) this error is 20% Hunt (1969) found the ultrasonic technique to be erroneous in only 4 out of 118 cases. Identical and reliable results were obtained by Pystynen et al. (1968) who used the visual (A-scan method) and auditory ultrasonic method in their studies.

Wood et al. (1970) compared the results obtained by the Doppler method with those obtained by a portable scintillation scanner with 113m Indium. They reported that the results given by the Doppler technique were false-negative in 28.5% According to their results, isotope study is 80% reliable compared with radiological examination. Hakim (1970) made a comparative study of the Doppler flowmeter and radioactive carbon monoxide and the gamma camera. Of a series of 100 patients, 50 underwent a caesarean section which confirmed the placental site.

Scanning or a gamma camera and various radioactive tracers can be used for isotope placentography The results obtained by the gamma camera are more accurate if both an antero-posterior and lateral exposure are made. The irradiation dose to the fetus is only an average of 5 mrad with the dosage used by us (Lavine, 1967)

The Doppler flowmeter study is safe and easy It provides by way of additional information other sound effects based on the blood flow Ex-

Table II *The 15 cases in which the position of the placenta determined by Doppler flowmeter and gamma camera differed*

The symbols are the same as in T ble I

Case	D gn	Reg	
		Doppler	Gamma camera
7		IV	I
12		II	I
38		II	I (III)
41		II	III (IV)
48		I	IV
5		I	II
58		I	III
61		I	IV
64		II (IV)	I
65	×	II	IV
71		II	I
78		I	II
81		I	II
92		I	II
96		I	II
		15	15

N m l pr g a y
M ld p l mp a
S e p e l mps

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HEPATIC BLOOD FLOW DURING LATE PREGNANCY

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Abstract. The liver blood flow in non-pregnant women (15 cases) and in late pregnancy (20 cases) was studied by colloidal heat-denatured serum albumin labelled with ^{131}I. The dose used was 10 µCi. The results were evaluated visually by gamma camera or two collimated detectors, one of which was directed to the vessels of the thigh and the other placed over the right lobe of the liver. In addition, blood samples were drawn at fixed intervals and their radioactivity was measured. Using previously developed mathematical formulae, the half-time of plasma activity was found to be 2.0 ± 0.4 min in the control group, 2.0 ± 1.1 min in normal pregnancy and 3.0 ± 1.1 min in toxaemia. Because of the great individual variation no significant changes between the groups were demonstrable. The isotope diagnostic method used was found to be suitable for measurement of the hepatic blood flow of patients with stable liver disease, but because of the changing vital functions and the individual variations during pregnancy this method does not bring out the changes between non-pregnant cases and late pregnancy.

Hepatic blood flow has been found to change in various diseases affecting the liver, e.g. cirrhosis of the liver, hypertension and metastasized carcinoma. Bromsulphalein was used first in studies of these groups of patients (Bradley et al., 1952), later indocyanine green (Caesar et al. 1961) and most recently radioactive isotopes. In the first isotope study colloidal chromic phosphate was employed by Dobson et al. (1952). The unreliability of this substance was compensated by colloidal gold (Vetter et al., 1954). Halpern et al. (1959) described a heat-denatured albumin colloidal complex labelled with ^{131}I (CA^{131}I). The measurement of total hepatic blood flow by CAI correlated well with the hepatic flow results obtained with indocyanine green, and this suggested that the method does indeed measure liver blood flow (Shaldon et al., 1961).

The isotope technique was applied in our study by using colloidal heat-denatured serum albumin labelled with ^{131}I tracer. We used this technique to study possible differences in the blood circulation in non-pregnant women and in the late pregnancy. The size of the particles of the tracer used is approx. 250–400 Å. As such it does not penetrate the placenta and is thus comparable in its radiation load with RIHSA which is considered to be a tracer harmless to the fetus at the end of the pregnancy.

MATERIAL

The series consisted of 5 normal pregnant women and 15 with mild pre-eclampsia who were examined between the 38th and 40th week of pregnancy. The control series comprised 15 patients of which cases 7, 8 and 10 had legal abortions for psychiatric indications in the 12th week of pregnancy. The other cases were patients with genital carcinomas. Liver scanning performed later in the course of their disease revealed pathological uptake in the liver of cases 9, 11 and 12. The haematocrit value of each patient examined was determined on the day of examination.

METHOD

Colloidal heat-denatured human serum albumin labelled with ^{131}I (Albumotope, I-H 131) was the substance used. The patient was given an intravenous injection of 10 µCi of the material in a volume of 0.5 ml. Blood samples (about 5 ml) were taken almost continuously from the antecubital vein of one side. The times at which the samples were taken were noted carefully. Activity was counted in the centrifuged plasma of each sample by an automatic gamma sample counter (Wallac-Klinner 651).

The plasma background caused by free radioiodine is deducted from the measured results. The value to be deducted is obtained from a 15–20 min sample. All the values were calculated in units of counts/min ml. The

substance used was not sufficient to indicate clearly observable differences in the gamma-camera picture series. The nephrography apparatus gave curves comparable with the half time for activity but nothing quantitative can be said about their significance in the individual cases.

DISCUSSION

This method of measuring liver blood flow without hepatic vein catheterisation assumes that the colloid is extracted completely in one passage through the liver. The tracer is removed by Kuppfer cells and the lower extraction could be due to the fact that blood bypasses the sinusoids containing Kuppfer cells due to the impairment of Kuppfer cell function or to a reduction in the number of Kuppfer cells lining each sinusoid (Shaldon et al., 1961). The tracer used has many advantages according to Halpern (1967). It is without any toxicity or allergenicity the size of its particles is uniform and constant, it is rapidly and completely metabolised by the Kuppfer cells, which prevents any lengthy accumulation of inert substances in the hepatic parenchyma. It can be used both for measuring hepatic blood flow and for investigating the phagocytic function of the reticulo-endothelial system. The amount of substance used by us corresponded to the isotope dose utilised for localisation of the placenta, which is regarded as safe for the fetus (Laakso & Pulkkinen, 1965). In concurrent [illegible]A and [131] I Rose Bengal studies Blaha et al. (1969) determined both the liver blood flow and polygonal cell function. Among other things, they found that the blood flow of patients with cancer of the liver remains unchanged when cell function decreases. Cases 9, 11 and 1[illegible] our series, showed the same finding. Ylöstalo et al. (1967) established that the hepatobiliary function of toxaemic patients in the puerperium is similar to that in normal pregnancy when studied by the radioactive [131] I Rose Bengal test. Our studies showed no difference in the half-time of the tracer in normal and toxaemic pregnancies compared with the control series. As the tracer employed, colloidal heat denatured human serum albumin labelled with [131] I metabolises in Kuppfer cells, it suggests that pregnancy does not affect the function of these cells. However Ikonen (1964) encountered hepatobiliary disturbances during pregnancy and attributed them to changes in the steroid metabolism. An alteration of the enzyme function of the liver may be an indirect factor in hepatic blood flow changes.

Great individual variations in total blood volume (65.3–142.4 ml/kg) in normal pregnancies at the same stage were established by Lund & Donovan (1967). They were seen in our series also. The method does not, therefore, seem suitable for analysis of group results, but may be useful for following the situation in an individual patient. This appeared (to be true) with the use of the nephrography apparatus. It is possible to compare the quantitative MHBF of individual patients with stable liver diseases such as cirrhosis, but the constantly changing vital functions and individual variations in pregnancy are not a reliable foundation for quantitative measurements by this method.

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Shaldon, S., Chiandussi, L., Guevara, L., Caesar, J. & Sherlock, S. The estimation of hepatic blood flow and intrahepatic shunted blood flow by colloidal heat

Table 1 *The cases divided into groups. Half-time values of Albumotope $H - {}^{131}I$ in serum ($T_{\frac{1}{2}}$) and the haematocrit values for each patient*

Case no	$T_{\frac{1}{2}}$ (min)	Haematocrit (%)
Controls		
1	1.9	41
2	2.5	48
3	2.1	43
4	2.0	40
5	2.8	44
6	1 7	35
7	2.1	37
8	2.8	42
9	1 7	45
10	1.5	38
11	1 7	34
12	1 4	36
13	1.9	41
14	[illegible]	43
15	2.2	44
15 Mean	2.0 ± 0.4	
Toxaemia		
16	1.9	39
17	1 8	43
18	1.6	35
19	2.3	38
20	3.5	42
21	3.5	43
22	2.9	40
23	3.0	44
4	2.4	38
25	4.2	43
26	2.9	42
27	5.8	39
28	4.3	44
29	[illegible]	40
30	2.5	43
15 Mean	3.0 ± 1 1	
Normal pregnancy		
31	3.2	37
32	3.5	32
33	0.8	42
34	1.2	38
35	1.6	37
5 Mean	2.0 ± 1 1	

calculated values were entered on semi-logarithmic scale against time. A descending straight line is normally obtained from which the activity of the plasma at zero time is extrapolated. The half-time ($T_{\frac{1}{2}}$) of plasma activity was noted. The following results were calculated in the manner presented by Halpern (1967):

The equation of the straight line plotted on semi-logarithmic paper may be written in the form

$$N_t = N\ e^{-kt}$$

in which N = activity of plasma after the time t (counts/min/ml) N = activity of plasma extrapolated from the observation points at time 0, t = the time (min) and k is a constant which characterises liver clearance.

Hence

$$k = \frac{0.693}{T_{\frac{1}{2}}}$$

The volume of plasma (PV) can be calculated as follows in accordance with the mixing principle:

$$PV = \frac{\text{activity of the standard dilution injection volume}}{\text{activity of the sample corrected to the zero time}}$$

Further the volume of total blood (TBV) is obtained by means of the haematocrit value (PCV):

$$TBV = PV \times \frac{100}{100 - (PCV \times 0.91)}$$

Hepatic flow is obtained by multiplying TBV by k if it is assumed that the colloidal hepatic extraction is 100%. This is not so, however and the error is under 10% (Shaldon et al., 1961). Hepatic flow calculated in the above manner without taking hepatic extraction into consideration has been called minimal hepatic blood flow (MHBF). Measurement of MHBF has been considered to suffice, especially as the measurement of hepatic extraction is relatively difficult.

We tried to measure hepatic activity as a function of time externally using both a gamma camera and a nephrography apparatus, and concurrently with the taking of the blood samples. An ordinary nephrography apparatus including two collimated detectors, corresponding pulse height analysers and recorder (Wallac) proved to be the most suitable. One of the two detectors was directed to the great vessels of the thigh (blood activity) and the other was placed over the right lobe of the liver (liver activity).

RESULTS

The results obtained are given in Table I. The half-times of plasma activity displayed such a great deviation in the individual cases of all the groups that significant differences could not be demonstrated between the groups. The half-time was fastest in the control group (2.0 ± 0 4 min) and in normal pregnancy (2.0 ± 1 1 min) and slowest in toxaemia (3 0 ± 1 1 min)

The total blood volume calculated from the half-time and the haematocrit value was 68 ± 4 ml/kg in the control group 50 ± 25 ml/kg in normal pregnancy and 69 ± 4 ml/kg in toxaemia. It was possible to calculate MHBF according to the same principles. It was 76 ± 10 ml/kg in the control group 18 0 ± 4 0 ml/kg in normal pregnancy and 18 ± 7 0 ml/kg in toxaemia. The deviation was so great in each of the investigation groups that no significant differences were demonstrable. The amount of the

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THE USE OF ULTRASONIC BIPARIETAL DIAMETER MEASUREMENT OF THE FETUS IN ASSESSING GESTATIONAL AGE

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Abstract. In 178 pregnant patients there was either inability to recall the date of the last menstrual period, or there was clinical discrepancy between uterine growth and calculated duration of pregnancy. The author attempts to forecast the expected date of delivery by ultrasonic measurement of fetal biparietal diameter. When the 40th week was considered as mean value, 96% of the patients delivered between the 37th and the 43rd week in the control group versus 84% in the study group. If the 41st week is taken as the mean value the corresponding figures are 94% and 91% respectively.

It is generally admitted that prolonged pregnancy ($N = 282$ days $\pm 2\sigma$) as well as premature delivery may be the cause of fetal distress (1, 2, 3). Therefore, in order to avoid unnecessary morbidity obstetricians would like to know with certainty the fetal development at any given period of gestation. When the pregnant woman is able to recall her last menstrual period (L.M.P.) or the day of fertilization, it is relatively easy to estimate the gestational age and to predict the expected date for delivery with a reasonable degree of accuracy (1, 5, 6).

However in our antenatal outpatient department, approximately 10% of the patients cannot fix a precise date for their last menstrual period (L.M.P.) and in another 10% there is a discrepancy between the fetal age as calculated from the L.M.P. and the clinical findings during the course of pregnancy. Commonly two ancillary methods are used to evaluate fetal age: radiological examination of bone development and ultrasonic fetal measurement.

Fetal growth is assessed routinely by ultrasonic measurement in our clinic and the normal fetal growth rate from the 14th to the 40th week of pregnancy has already been established by ultrasonic biparietal measurement (4). Therefore, we prefer to employ this harmless procedure for assessing gestational age.

METHODS AND MATERIALS

Between January 1, 1969 and August 31, 1970, 1 119 pregnant women were submitted to ultrasonic examination (A and B-mode) at the Diagnostic Ultrasound Department of the Gynecological Unit. In the present retrospective study 42 patients in whom labour was induced are excluded.

In the 1 077 remaining patients, 104 were unable to establish a firm date for their L.M.P. (Group A). In 86 other patients, there was a discrepancy between the gestation period as judged by the L.M.P. and the physical examination (Group BC). In these two groups, comprising 190 cases, the gestational age needed to be established by ultrasonic fetal measurements.

Of the 190 cases involved, 12 cases were excluded because of premature delivery and a birth weight of less than 2 400 g. The remaining cases (178) were then divided into two groups: those women who gave birth to babies of at least 2 500 g and secondarily those delivering babies of more than 2 600 g and 47 cm in length. In most cases biparietal values were available between the 14th and the 40th week but, in order to render our series homogeneous, only the first measurement was considered. The expected date for delivery was estimated from this first biparietal measurement as follows:

1. the biparietal measurement is referred to the mean value on our growth curve and the fetal age is read in weeks on the gestational axis;

– the first day of the week corresponding to the fetal age determined following the method described in (1), is referred to the date estimated by ultrasonic examination on an Ortho Birth Calculator (from Ortho Diagnostics Lab.) where the expected delivery date is indicated by an arrow;

3. the actual delivery date is compared to this "ultrasonic" fetal age and is noted in weeks. Thus, in the present study the week number written on the Birth Calculator (for example 39 weeks) would indicate the 39th

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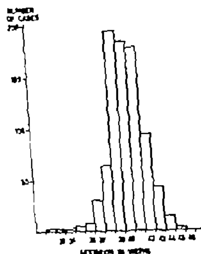


Fig. 4. Case distribution in relation with gestational week. Control group (L.M.P. known) infant ≥ 2 500 g.

3 days. As shown in Table I, the mean delivery occurred at 41 weeks in group A_1 and A_2.

2. *Group B (discrepancy between L.M.P. and clinical age).* After eliminating 3 cases in which the infant's birth weight was less than 2 500 g this second group was also subdivided: Group B_1 comprises 83 cases with infants weighing at least 2 500 g and Group B_2 includes 80 of the 83 cases where the newborn weighed at least 2 600 g and was at least 47 cm in length.

Groups C_1 and C_2 include the same cases as groups B_1 and B_2 but, in group C, the gestational age is calculated from the L.M.P. instead from ultrasonic measurement as in group B. The number of cases for each gestational age at delivery is represented in Fig. 2, in white columns for group B_1 and in black columns for group C_1. All cases for which gestational age was determined by ultrasonic measurements ($A + B_1$) are grouped in Fig. 3.

3. *Control group (L.M.P. known).* The mean gestational age is generally considered to be 281 or 282 days (40 *completed* weeks or during the 41st week, according to the Birth Calculator used for this study). In order to verify this data in our population, the gestational age at confinement was computed in a parallel series of 827 patients delivered during the present study who were able to recall the date of their L.M.P. in whom 823 had infants weighing 2 500 g or more (Fig. 4).

B. *Case distribution in relation to the mean gestational age*

From the practical point of view it is important to know not only the mean gestational age but also the time limits during which most of the deliveries supervened. Only the largest groups (A_1, B_1 and C_1) were considered for this purpose.

Since the theoretical mean gestational age is situated in the 41st week and since our values of the control group are in the 40th week both durations were considered as reference. The percent age difference between the number of cases in the ultrasonic group (A and B_1) and in the control group is noted for each mean gestational age (40th and 41st week) (Table II).

Table II. *Case distribution in relation to the mean age value*

Deviation (weeks)	Group A		Group B_1		Group C_1		Groups A_1 B_1		Control group		Difference control a. A_1 B_1
1 (41 ± 1)	38	40.0	46	55.4	41	49.4	84	43.8	457	55.5	11.7
(40 ± 1)	36	43.4	48	58.1	39	46.9	92	51.7	557	67.6	15.9
2 (41 ± 2)	65	67.4	64	77.1	55	66.2	129	72.5	694	84.3	11.8
(40 ± 2)	61	64.5	67	81.3	54	66.3	127	71.3	721	86.3	15.0
3 (41 ± 3)	83	86.3	79	95.2	64	77.1	162	91.0	777	94.4	3.4
(40 ± 3)	73	79.6	75	91.3	71	85.1	149	83.7	793	96.3	12.6
Total no. of cases	95	100.0	83	100.0	83	100.0	178	100.0	823	100.0	

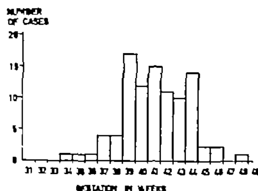


Fig 1 Case distribution in relation to gestational week. Group A_1 (L.M.P unknown) infant ⩾ 2 500 g.

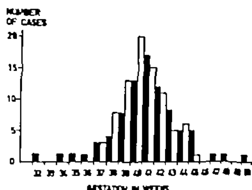


Fig 2 Case distribution in relation to gestational week. Group B_1 C_1 (L.M.P known but discrepant with clinical age) (infant ⩾ 2 500 g) White columns represent Group B_1 (age calculated by ultrasonic measurement). Black columns represent Group C_1 (age calculated by L.M.P.)

week of pregnancy that is, a gestational age situated between 38 completed weeks and 38 weeks and 6 days.

This same method for calculation was applied to the group BC (L.M.P questionable): group B includes those results obtained from the biparietal criterion, whereas group C includes the results obtained from the L.M.P criterion.

RESULTS

A. *Number of births in relation with gestational age*

1 *Group A (L.M.P unknown)* Group A comprises 104 cases but 9 were excluded because of premature delivery and a birth weight of under 2 500 g. Group A_1 comprises the remaining 95 cases where the birth weight was 2 500 g or more.

Group A_2 comprises 91 of these cases in which the birth weight was 2 600 g or more and the *fetal length 47 cm or more. This further subdivision* was chosen to facilitate comparison with the findings of other workers, based on a very large series (5) Fig. 1 shows the number of births in respect to the gestational age as calculated from ultrasonic measurement. As mentioned, the mean fetal age at delivery is calculated in *uncompleted* pregnancy weeks.

To facilitate comparison with the results of other who expressed the fetal age in days post L.M.P our values are also expressed in days. For example, 40 weeks would be equivalent to 277 ±

Table I

		Average gestational duration			
		Weeks[b]		Days[c]	
Groups[a]	N of cases	*m*	S.D	*m*	S.D
A_1	95	41.1	2.5	284 ± 3	~ 17.5
A_2	91	41.2	2.2	285 ± 3	~ 15.4
B_1	83	40.5	1.8	280 ± 3	~ 12.6
B_2	80	40.6	1.8	281 ± 3	~ 12.6
$A_1 + B_1$	178	40.8	2.2	282 ± 3	~ 15.4
C_1	83	41.0	2.8	284 ± 3	~ 19.6
C_2	80	41.0	2.7	284 ± 3	~ 18.9
Control	823	40.0	1.7	277 ± 3	~ 11.9

[a] A L.M.P unknown. B, C: discrepancy L.M.P age : clinical age. A, B gestational age determined by ultrasonic measurement. C: gestational age calculated from L.M.P Control gestational age calculated from L.M.P
A_1, B_1 C_1 and Control infant > 2 500 g.
A_2, B_2 and C_2 infant > 2 600 g and 47 cm.
[b] Uncompleted week.
[c] Number of days calculated from week value (ex. 40th week = 277 ± 3 d.).

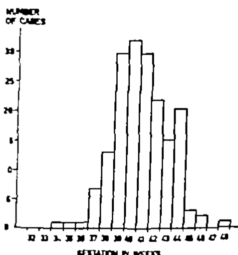


Fig 3 Case distribution in relation to gestational week. Groups $A_1 + B_1$ or all cases in which gestational age was *computed by ultrasonic measurements) infant* ⩾ 2 500 g.

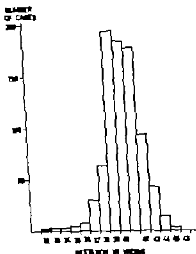


Fig. 4. Case distribution in relation with gestational week. Control group (L.M.P. known) infant ≥ 2 500 g

3 days. As shown in Table I, the mean delivery occurred at 41 weeks in group A_1 and A_2.

2. *Group B (discrepancy between L.M.P. and clinical age).* After eliminating 3 cases in which the infant's birth weight was less than 2 500 g this second group was also subdivided. Group B comprises 83 cases with infants weighing at least 2 500 g and Group B_2 includes 80 of the 83 cases where the newborn weighed at least 2 600 g and was at least 47 cm in length.

Groups C_1 and C_2 include the same cases as groups B_1 and B_2 but, in group C, the gestational age is calculated from the L.M.P. instead from ultrasonic measurement as in group B. The number of cases for each gestational age at delivery is represented in Fig. 2, in white columns for group B_1 and in black columns for group C_1. All cases for which gestational age was determined by ultrasonic measurements ($A_1 + B_1$) are grouped in Fig. 3.

3. *Control group (L.M.P. known).* The mean gestational age is generally considered to be 281 or 282 days (40 *completed* weeks or during the 41st week, according to the Birth Calculator used for this study). In order to verify this data in our population, the gestational age at confinement was computed in a parallel series of 827 patients delivered during the present study who were able to recall the date of their L.M.P. in whom 823 had infants weighing 2 500 g or more (Fig. 4).

B. *Case distribution in relation to the mean gestational age*

From the practical point of view it is important to know not only the mean gestational age but also the time limits during which most of the deliveries supervened. Only the largest groups (A, B and C_1) were considered for this purpose.

Since the theoretical mean gestational age is situated in the 41st week and since our values of the control group are in the 40th week both durations were considered as reference. The percentage difference between the number of cases in the ultrasonic group (A and B_1) and in the control group is noted for each mean gestational age (40th and 41st week) (*Table II*).

Table II. *Case distribution in relation to the mean age value*

	Deviation (weeks)	Group A_1		Group B_1		Group C_1		Groups A_1+B_1		Control group		Difference control v. A_1+B_1 %
					%		%		%		%	
1	(41 ± 1)	38	40.0	46	55.4	41	49.4	84	43.8	457	55.5	11.7
	(40 ± 1)	46	48.4	43	50.1	39	46.9	92	51.7	557	67.6	15.9
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	(40 ± 2)	61	64.5	67	81.3	54	66.5	127	71.3	721	86.3	15.0
3	(41 ± 3)	83	86.3	79	95.1	64	77.1	162	91.0	777	94.4	3.4
	(40 ± 3)	75	79.6	75	91.3	71	85.1	149	83.7	793	96.3	12.6
	Total no. of cases	95	100.0	83	100.0	83	100.0	178	100.0	823	100.0	

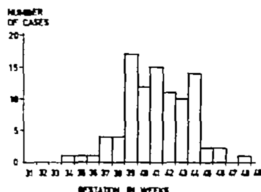


Fig 1 Case distribution in relation to gestational week. Group A_1 (L.M.P unknown) infant $\geq$2 500 g.

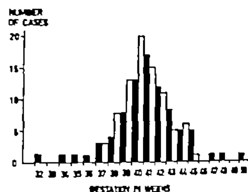


Fig 2 Case distribution in relation to gestational week. Group B_1 C_1 (L.M.P known but discrepant with clinical age) (infant $\geq$2 500 g) White columns represent Group B_1 (age calculated by ultrasonic measurement). Black columns represent Group C_1 (age calculated by L.M.P.)

week of pregnancy that is: a gestational age situated between 38 completed weeks and 38 weeks and 6 days.

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To facilitate comparison with the results of other who expressed the fetal age in days post L.M.P., our values are also expressed in days. For example, 40 weeks would be equivalent to 277±

Table I

Groups	No. of cases	Average gestational duration			
		Weeks[b]		Days[c]	
			S.D	x	S.D
A	95	41 1	2.5	284±3	~17.5
A	91	41.2	2.2	285±3	~15 4
B_1	83	40.5	1.8	280±3	~12.6
B_2	80	40.6	1.8	281±3	~12.6
A +B_1	178	40.8	2.2	282±3	~15 4
C_1	83	41.0	2 8	284±3	~19 6
C_2	80	41.0	.7	284±3	~18.9
Control	823	40.0	1 7	277±3	~11.9

A L.M.P unknown. B, C. discrepancy L.M.P age clinical age. A, B. gestational age determined by ultrasonic measurement. C: gestational age calculated from L.M.P
Control gestational age calculated from L.M.P
A_1, B_1 C_1 and Control. infant >2 500 g.
A_2, B_2 and C_2 infant >2 600 g and 47 cm.
[b] Uncompleted week.
Number of days calculated from week value (ex 40th week = 277±3 d.).

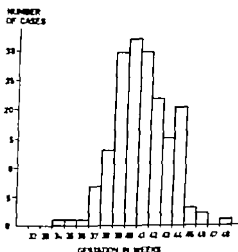


Fig 3 Case distribution in relation to gestational week. Groups A_1 + B_1 or all cases in which gestational age was computed by ultrasonic measurements) infant $\geq$2 500 g.

Acta Obstet Gynec Scand 50 183–192, 1971

OXYGEN TENSION ACID-BASE STATUS AND ELECTROLYTES IN HUMAN AMNIOTIC FLUID

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Abstract. Determinations of oxygen tension, acid–base and electrolyte components in amniotic fluid were made in 199 cases of early pregnancy (gestational age 11 24 weeks) and in 107 cases of late pregnancy (gestational age 26–40 weeks). The values of P_{O_2}, pH, sodium, chloride and osmolality decreased with advancing gestation, while those of P_{CO_2} and potassium increased. Bicarbonate values are higher in the 12th week than later on in pregnancy. The values of calcium and lactate were fairly constant. Total protein was higher in the 5th month than in the earlier and later periods of pregnancy. In late pregnancy the P_{O_2} values in amniotic fluid were positively correlated to maternal values of P_{CO_2} and to base excess.

Since the first reports on P_{O_2} (50), P_{CO_2} (51), and on pH in amniotic fluid (6.) there have been many publications on these subjects (3 5 10, 18 22, 29 39 40, 41 42, 43, 44 45, 59 60, 61).

Sjöstedt et al. (51) reported a mean value for P_{O_2} of 11.0 mmHg in early pregnancy and of 7.0 mmHg in late pregnancy. Other investigators reported mean values for P_{O_2} in late pregnancy varying between 8.8 mmHg and 27.6 mmHg (5, 18, 39 40, 41 59 60, 61). Reported mean values for P_{CO_2} varied between 41.1 mmHg and 55.0 mmHg in early pregnancy and between 48.3 mmHg and 58.0 mmHg in late pregnancy (3, 5 10, 22, 42, 43 44 45 51). For pH mean values varying between 7.10 and 7.23 in early and between 6.98 and 7.11 in late pregnancy were reported (3, 5, 22, 29 42, 43 44, 45 51).

With reference to detailed investigation into problems concerning the technique of sampling amniotic fluid and in the analytic technique for determinations of P_{O_2}, P_{CO_2} and pH in this fluid, performed in our Departments (16, 17), it may be suspected that mean values for P_{O_2} higher than those given by Sjöstedt et al. are erroneous and that P_{CO_2} values obtained with the Astrup equilibration technique are often too high.

The present report describes the values of P_{O_2}, P_{CO_2} and pH in amniotic fluid found in early and late pregnancy. The electrolyte composition of amniotic fluid was also studied and compared with that of maternal serum. Maternal arterial blood P_{O_2} and acid–base status were determined in some cases and compared with P_{O_2} and acid–base status in amniotic fluid.

MATERIAL AND PROCEDURE

The material consisted of an early pregnancy series of 199 cases (11 24 weeks of gestation) and a late pregnancy series of 107 cases (26–40 weeks of gestation).

In the early pregnancy series, the amniotic fluid samples were drawn from women admitted into hospital for legal abortion. This series was subdivided into sub-groups according to the gestational age. In the sub-group 11 12 weeks the puncture was experimental and in the sub-group 13–24 weeks the puncture constituted part of the procedure for the induction of the therapeutical abortion. The pregnancy was apparently normal in 185 of these cases and probably abnormal in 14.

In the late pregnancy series, 134 samples were drawn for diagnostic reasons from 94 iso-immunized mothers, and 13 for experimental reasons from mothers with jaundice, toxaemia or hydramnios. In iso-immunized mothers, amniocentesis was often made at regular intervals. Therefore many of these mothers were represented by samples at different stages of gestation. When findings in samples from these cases were related to the severity of haemolytic disease of the newborn, only one sample in each case, drawn within 1 week of delivery was accepted. On the other hand, when the colour index, calculated from the spectral absorption curve (19 23) was related to other findings in amniotic fluid all samples were included.

In order to discover if any of the parameters under investigation were correlated with the severity of haemolytic disease in the infants, the whole series was subdivided into the following sub-groups, with reference to the infant status at birth:

III. COMMENTS AND CONCLUSION

When compared with the average gestational week at confinement in the control group where the first day of the LMP is known the average week is slightly more elevated for all other groups the expected date of delivery calculated by means of the ultrasonic method, when translated from weeks into days is the same as for the generally admitted mean duration of pregnancy i.e. 287 days after the 1st day of the LMP (group $A_1 + B_1$) The standard deviation is greater when the gestational age is derived from the ultrasonic measurements than in the control group It is also greater in our control group than in some other reports in the literature 11.9 days versus 11 days reported by Rosa (1) but Sterky reports 13.5 days as standard deviation (6) The mean gestational duration in Group C is not very different from that of the other groups but the standard deviation is much greater than for those cases (group B) where the gestational age was ultrasonically calculated (19 6 versus 12.6)

Finally if we consider the classical average gestational age, i.e 282 days ($\sigma = 11$ or 13.5 days) (1 6) the comparison with the ultrasonically calculated age differs only by a larger standard deviation of 15 4 days (Table 1 group $A_1 + B_1$) Moreover 91% of our studied cases delivered within 7 weeks of the expected date of delivery as compared with 96% in large series with LMP known.

The prediction of expected confinement date is thus nearly as good in patients who cannot recall their LMP and examined by ultrasound as in patients who remember their LMP

ACKNOWLEDGEMENT

I am grateful to Bertil Sundén, M.D (Lund) who initiated me in diagnostic ultrasound.

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Table I. *Oxygen and carbon dioxide tension and hydrogen ion concentration in amniotic fluid at different gestational periods in apparently normal patients and in patients with varying degrees of iso-immunization*

Pregnancies with gestational age (g.a.) of 27 to 40 weeks were subdivided into 4 sub-groups (see Material and Procedure). All samples in sub-groups 2–4 were drawn within 1 week of delivery. In the combined series all samples are presented from all patients with an Apgar score 7 or more at birth, without taking into consideration the degree of haemolytic disease

Sub-groups	P_{O_2}			P_{CO_2}			pH		
		Mean ± S.E.M. (mmHg)	Range		Mean ± S.E.M. (mmHg)	Range		Mean ± S.E.M.	Range
12 weeks g.a.	12	11.8±1.0	2.4–15.8	10	47.8±0.9	42.3±52.5	12	7.32±0.025	7.18–7.44
13–24 weeks g.a.	154	11.2±0.3	2.6–21.7	149	47.0±0.3	39.6–56.0	141	7.17±0.004	7.04–7.29
27–40 weeks g.a.									
Sub-group 1	25	5.7±0.6	0.0–11.9	25	53.8±0.8	43.7–63.2	26	7.11±0.010	7.02–7.23
Sub-group 2	16	5.2±0.6	0.0–10.0	16	53.8±0.5	51.2–58.1	14	7.08±0.012	7.00–7.15
Sub-group 3	9	6.3±1.0	3.3–12.1	10	52.2±1.0	46.1–56.3	10	7.12±0.012	7.07–7.20
Sub-group 4	3	5.8±2.1	3.3–10.0	4	53.4±1.9	48.9–58.1	3	7.13±0.037	7.09–7.20
Combined series	67	6.0±0.3	0.0–14.0	100	53.1±0.4	43.7–63.2	94	7.11±0.004	7.00–7.23

of iso-immunization are given. Individual values from patients with abnormalities apart from iso-immunization or with infants born with an Apgar score less than 7 are presented in Table II. Two early pregnancy patients with no other obvious abnormalities than a P_{O_2} value (Case 2, Table II) or a P_{CO_2} (Case 12, Table II) deviating by more than 3 standard deviations from the mean in the normal series, are included in Table II. The early pregnancy series was divided into two sub-groups having a gestational age of 12 weeks and 13–24 weeks, respectively. They did not differ significantly in P_{O_2} or P_{CO_2} values, but the pH values were significantly higher (Student's test $t=8$) in the sub-group 12 weeks than in the sub-group 13–24 weeks. Within the sub-group 13–24 weeks, no significant difference in the values of P_{O_2}, P_{CO_2} or pH were found when the results from different weeks were compared. This was also true when the results from different months in the late pregnancy series were analysed statistically. Cases from the 13th–24th weeks were therefore combined in one group and cases from the 27th–40th weeks in another. When the results from the 13th–24th weeks group were compared with the normal late pregnancy group it was found that P_{O_2} and pH values were significantly higher in the former group while P_{CO_2} was significantly lower ($t=8$, 9 and 6, respectively). The P_{O_2}, P_{CO_2} and pH values in pregnancies complicated by haemolytic disease of the foetus did not deviate significantly from those in the normal late pregnancy sub-group. This was the case regardless of the severity of haemolytic disease as judged from the condition of the newborn (sub-groups 2–4), the need for exchange transfusions, or the peak at 450 nm in the spectral absorption curve. Thus, haemolytic disease of the newborn did not influence the P_{O_2}, P_{CO_2} or pH values of amniotic fluid when the infant was alive and vigorous. The results from the combined late pregnancy series may therefore be reasonably considered as normal values for P_{O_2}, P_{CO_2} and pH.

Electrolyte components

The electrolyte composition of maternal serum in the different groups of iso-immunization did not differ significantly from that of the normal late pregnancy group. It was also found that the electrolyte composition of maternal serum was fairly constant throughout pregnancy. The only statistically significant differences in the mean values found between early and late pregnancy cases were for calcium (diff. +0.2 mmol/l, $t=5.24$), magnesium (diff. 0.07 mmol/l, $t=2.01$) and chloride (diff. +1.6 mmol/l, $t=2.42$). However these differences were too small to be of practical importance. Mean values for serum electrolytes (Table III) are therefore given for the whole gestational period. Some of the electrolytes in amniotic fluid showed significant variations with gestational time, while no variations due to different degrees of haemolytic disease of the infants were noted. Therefore the results in Table III are subdivided in different gestational periods but not in different immunization groups. It is well known that there is a correlation between

1. Direct Coombs test negative, 32 samples from 26 cases.
2. Direct Coombs test positive, cord blood haemoglobin (Hb) above 12.0 g/100 ml, 48 samples from 38 cases.
3. Direct Coombs test positive, cord blood Hb 8.0–12.0 g/100 ml, 41 samples from 21 cases.
4. Direct Coombs test positive, cord blood Hb below 8.0 g/100 ml, 11 samples from 7 cases.
5. Intra-uterine foetal death due to haemolytic disease 2 samples from 2 cases.

With reference to the need for exchange transfusion of the infants, the series of 132 samples from 92 cases with living infants at birth was subdivided into the following sub-groups.

A. *No exchange transfusion, 49 samples from 39 cases.*
B. 1–2 exchange transfusions, 42 samples from 27 cases.
C. 3–7 exchange transfusions, 41 samples from 26 cases.

With reference to the peak at 450 nm in the spectral absorption curve for amniotic fluid (zones according to Liley) (19 23) the complete series of 134 samples was subdivided into the following sub-groups

I Mild zone, 46 samples.
II Moderate zone 61 samples.
III Severe zone, 27 samples.

All samples of amniotic fluid were drawn int sterile siliconized all-glass syringes using an anaerobic technique de scribed earlier (16). Arterial blood samples were drawn into heparinized, siliconized all-glass syringes by puncture of the femoral artery immediately after amniocentesis. Before arterial puncture the mothers had been lying in the supine body position for 10–20 min, during amniocentesis. All syringes were immediately sealed with a metal cap and stored in iced water until analyses were performed. All amniotic fluid samples with visible contamination of blood were rejected from the investigation (16, 17).

Before amniocentesis 50 cases in the early pregnancy series were examined for foetal heart activity using an ultrasound technique (Doptone Foetal Pulse Detector Smith and Kline. *Instrument Company). In the late pregnancy series, all cases* were examined for foetal heart activity with a stethoscope before and after amniocentesis.

The procedure and its objective was described to the mothers. They were invited t participate and given free choice. Thus, free and informed consent had been given in all cases. There were no complications resulting from the procedure.

ANALYTIC METHODS

P_{O_2}, P_{CO_2} and pH in amniotic fluid samples were analysed at 37.0±0.1 C with Radiometer electrodes (E 5046, E 5036 and G 297/G 2). All measurements were made i duplicate. The random error of a single determination was 0.45 mmHg for P_{O_2} at mean value of 9 8 mmHg, 0.73 mmHg for P_{CO} for pH at a mean value of 49.2 mmHg and 0.005 for pH at a mean value of 7 156. Details of the analytic procedure were reported earlier (16, 17).

P_{O_2} and pH in arterial blood samples were analysed with the same electrodes as amniotic fluid, but the P_{O_2} lectrode was calibrated with a gas mixture with a P_{O_2} of about 70 mmHg.

During the first period of the investigation, arterial blood P_{CO_2} was determined using the Astrup equilibration technique (46, 47). In these cases standard bicarbonate and base excess were calculated from the Siggaard Andersen nomogram. During the other period of the investigation, arterial blood P_{CO_2} was determined with a P_{CO_2} electrode. Standard bicarbonate and base excess were then calculated with Blood Gas Calculator (Type BGC 1 Radiometer). All determinations were made in duplicate with the same precautions as for analyses in amniotic fluid. The random error of a single determination was 0.8 mmHg for P_{O_2} at a mean value of 90 mmHg, 0.3 mmHg for P_{CO_2} with the P_{CO_2} electrode at a mean value of 31 6 mmHg and 0.005 for pH at a mean value of 7 40.

Determinations of sodium, potassium, calcium, magnesium chloride and osmolality in serum and amniotic fluid, as well as standard bicarbonate and total protein in serum, were made routinely at the Department of Clinical Chemistry Sodium, potassium and calcium were assayed by flame photometry (14). Magnesium was assayed by atomic absorption spectrophotometry (53). Chloride was assayed by a colorimetric method (55) Standard bicarbonate was determined by the Astrup equilibration technique (20). Total protein was assayed by an ultra-violet absorption spectrophotometry technique modified for dialysed serum (35). Osmolality was determined by measuring the depression of the freezing point with a Knauer Osmometer Some of these methods were modified slightly for routine work. The methodological errors for the analyses would correspond to the following coefficients of variation sodium 2.2%, potassium 2.9 calcium 2.2%, magnesium 5.7% chloride 1.6%, standard bicarbonate 4.3% total protein 2.1% and osmolality 2.0

Total protein in amniotic fluid was determined by a modified biuret method (8) The random error of a single determination was 0.009 g/100 ml at a mean value of 0.305 g/100 ml.

Values of total protein in serum and amniotic fluid are recalculated from values in g/100 ml to mmol/l by multiplying by the van Slyke factor 2.43 (56). This would only give a rough estimation of mniotic fluid total protein in mmol/l as the van Slyke factor has not been proved to be valid in this fluid.

Lactate in amniotic fluid was determined in duplicate by a colorimetric method (4) modified by Ström (54). The random error of a single determination was 0.3 mmol/l at a mean value of 9 4 mmol/l.

Actual bicarbonate in amniotic fluid was calculated from the values of P_{CO_2} and pH by means of the *Henderson-Hasselbalch* equation (15). The mean values for P_{CO_2} and pH in different gestational periods were plotted in a Siggaard-Andersen nomogram (46) revised for the values of *pK* and αCO_2 in amniotic fluid (15). Lines were drawn with a slope given by Rooth et al. (42). The same slope of the buffer line in this nomogram was found t be valid for amniotic fluid by the present uthors in an earlier investigation (17). In this way the mean values for actual bicarbonate in amniotic fluid were recalculated to 'standard bicarbonate' values.

RESULTS

Oxygen tension, carbon dioxide tension and pH

In Table I mean values of P_O, P_{CO_2} and pH at different gestational periods and in the different groups

Table I. *Oxygen and carbon dioxide tension and hydrogen ion concentration in amniotic fluid at different gestational periods in apparently normal patients and in patients with varying degrees of iso-immunization*

Pregnancies with gestational age (g.a.) of 27 to 40 weeks were subdivided into 4 sub-groups (see Material and Procedure). All samples in sub-groups 2–4 were drawn within 1 week of delivery. In the combined series all samples are presented from all patients with an Apgar score 7 or more at birth, without taking into consideration the degree of haemolytic disease

Sub-groups	P_{O_2}			P_{CO_2}			pH		
		Mean ± S.E.M. (mmHg)	Range		Mean ± S.E.M. (mmHg)	Range		Mean ± S.E.M.	Range
12 weeks g.a.	12	11.8±1.0	2.4–15.8	10	47.8±0.9	42.3±52.5	12	7.32±0.028	7.18–7.44
13–24 weeks g.a.	154	11.2±0.3	2.6–21.7	149	47.0±0.3	39.6–56.0	141	7.17±0.004	7.04–7.29
27–40 weeks g.a.									
Sub-group 1	25	5.7±0.6	0.0–11.9	28	53.8±0.8	43.7–63.2	26	7.11±0.010	7.02–7.23
Sub-group 2	16	5.2±0.6	0.0–10.0	16	53.8±0.5	51.2–58.1	14	7.08±0.012	7.00–7.15
Sub-group 3	9	6.3±1.0	3.3–12.1	10	52.2±1.0	46.3–56.3	10	7.12±0.012	7.07–7.20
Sub-group 4	3	5.8±2.1	3.3–10.0	4	51.4±1.9	48.9–58.1	3	7.13±0.037	7.09–7.20
Combined series	87	6.0±0.3	0.0–14.0	100	53.1±0.4	43.7–63.2	94	7.11±0.004	7.00–7.23

of iso-immunization are given. Individual values from patients with abnormalities apart from iso-immunization or with infants born with an Apgar score less than 7 are presented in Table II. Two early pregnancy patients with no other obvious abnormalities than a P_{O_2} value (Case 2, Table II) or a P_{CO_2} (Case 12, Table II) deviating by more than 3 standard deviations from the mean in the normal series, are included in Table II. The early pregnancy series was divided into two sub-groups having gestational age of 12 weeks and 13–24 weeks, respectively. They did not differ significantly in P_{O_2} or P_{CO_2} values, but the pH values were significantly higher (Student's test $t=8$) in the sub-group 12 weeks than in the sub-group 13–24 weeks. Within the sub-group 13–24 weeks, no significant difference in the values of P_{O_2}, P_{CO_2} or pH were found when the results from different weeks were compared. This was also true when the results from different months in the late pregnancy series were analysed statistically. Cases from the 13th–24th weeks were therefore combined in one group and cases from the 27th–40th weeks in another. When the results from the 13th–24th weeks group were compared with the normal late pregnancy group it was found that P_{O_2} and pH values were significantly higher in the former group while P_{CO_2} was significantly lower ($t=8$, 9 and 6, respectively). The P_{O_2}, P_{CO_2} and pH values in pregnancies complicated by haemolytic disease of the foetus did not deviate significantly from those in the normal late pregnancy sub-group. This was the case regardless of the severity of haemolytic disease as judged from the condition of the newborn (sub-groups 2–4), the need for exchange transfusions, or the peak at 450 nm in the spectral absorption curve. Thus, haemolytic disease of the newborn did not influence the P_{O_2}, P_{CO_2} or pH values of amniotic fluid when the infant was alive and vigorous. The results from the combined late pregnancy series may therefore be reasonably considered as normal values for P_{O_2}, P_{CO_2} and pH.

Electrolyte components

The electrolyte composition of maternal serum in the different groups of iso-immunization did not differ significantly from that of the normal late pregnancy group. It was also found that the electrolyte composition of maternal serum was fairly constant throughout pregnancy. The only statistically significant differences in the mean values found between early and late pregnancy cases were for calcium (diff. +0.2 mmol/l, $t=5.24$), magnesium (diff. +0.07 mmol/l, $t=2.01$) and chloride (diff. +1.6 mmol/l, $t=2.42$). However these differences were too small to be of practical importance. Mean values for serum electrolytes (Table III) are therefore given for the whole gestational period. Some of the electrolytes in amniotic fluid showed significant variations with gestational time, while no variations due to different degrees of haemolytic disease of the infants were noted. Therefore the results in Table III are subdivided in different gestational periods but not in different immunization groups. It is well known that there is a correlation between

Table II *Oxygen tension, carbon dioxide tension hydrogen ion concentration and bicarbonate concentration in amniotic fluid in cases of abnormal or suspected abnormal pregnancy*

Case no	Gestational week	P_{O_2} (mmHg)	P_{CO_2} (mmHg)	pH	HCO_3^- (mmol/l)	Foetal heart activity	Comments
1	12	1.0	54.1	7.16	18.3	+	Uterine bleeding
2	12	0.0	53.9	7.33	27.1		Abnormally low P_{O_2}
3	14	5.8	50.3	7.02	19.5	+	*Uterine bleeding*
4	15	13.5		7.09			Discoloured amniotic fluid
5	15	8.3	54.0	7.16	18.2		Discoloured amniotic fluid
6	15	5.1	50.9	7.21	19.6	–	No foetal heart activity
7	15	3.7	48.7	7.18	17.3	–	No foetal heart activity
8	16	12.0		7.10			Discoloured amniotic fluid
9	16	1.0					Rubella during pregnancy
10	16	9.6	44.8	7.08	12.8	+	Discoloured amniotic fluid
11	17	4.[illegible]	48.8	7.04	1[illegible].7		Uterine bleeding
12	17	8.3	61.3	7.17	[illegible]1.2		Abnormally high P_{CO_2}
13	19	12.7	49.2	7.17	17.1		*Uterine bleeding*
14	20	3.3	45.1	7.14	14.7		Foetus small for dates
15	26	3.7	54.5	7.19	19.7	+	Intra-uterine foetal death 8 days later
16	28	3.6	51.3	7.11	15.6	+	Jaundice
17	29	9.6	49.9	7.22	19.7	+	Hydramnios
18	29	7.1	49.0	7.22	19.1	+	Toxaemia
19	30	2.0		7.10		–	Toxaemia. Foetal death
20	32	3.1	55.8			+	Jaundice
21	35	10.0	48.9	7.20	18.5	+	Apgar score 1
22	35	9.3	46.1	7.20	17.1	+	Apgar score 2
23	36	6.9	51.4	7.11	15.6	+	Jaundice
24	37	4.0	52.4			+	Apgar score 5
25	38	3.1	42.4	7.09	12.4	+	Jaundice Hydramnios
26	38	7.4	56.7	7.11	17.0	+	Jaundice
27	38	4.[illegible]	53.5	7.06	14.6	+	Jaundice
28	38		54.5	7.1	17.0	+	Toxaemia
29	38	0.0	52.2	7.14	17.1	+	Apgar score 6
30	38	7.5	53.9			+	Apgar score 6
31	38	5.3	52.0	7.04	13.6	+	Apgar score 5
32	38	2.1	55.2	7.11	16.6	+	Intra-uterine foetal death days later
33	39	2.9	51.2	7.02	12.7	+	Jaundice
34	40	6.4	48.3			+	Hydramnios
35	40		54.6	7.05	14.6	+	Jaundice
36	41	6.8	52.2	7.08	14.9	+	Toxaemia

amniotic fluid total protein content and severity of haemolytic disease in iso-immunized patients (38 57). Therefore only cases from the iso-immunized sub-group I were included in the figures for amniotic fluid total protein in Table III

During the whole gestational period sodium and calcium values were significantly higher in maternal serum than in amniotic fluid. During early pregnancy and in the 8th month potassium values in maternal serum were higher than in amniotic fluid. In the 9th and 10th months no difference was found in this respect. Before the 17th week magnesium concentrations were equal in both maternal serum and amniotic fluid. In later periods the amniotic fluid values were significantly lower than maternal serum values.

The chloride values in amniotic fluid were very similar to those of maternal serum in the 17th week and in the 10th month. From the 13th to the 36th week the amniotic fluid chloride values were significantly higher than the maternal serum values.

In Table III the actual bicarbonate values in amniotic fluid are given with maternal serum standard bicarbonate values. When the mean values for amniotic fluid P_{CO_2} and pH in the different periods of gestation were plotted into a Siggaard Andersen nomogram revised for amniotic fluid (see under Methods) the actual bicarbonate figures did not differ significantly from the figures for standard bicarbonate. Therefore, the actual bicarbonate figures in amniotic fluid were considered comparable to the

maternal standard bicarbonate figures. The comparison demonstrated that, in the 12th week, the bicarbonate concentrations in amniotic fluid and in maternal serum were very similar. In later stages of gestation, the amniotic fluid figures were significantly lower and fairly constant.

The concentrations of sodium, magnesium and chloride all showed a tendency to decrease towards the end of gestation. For potassium a reversed tendency was noted. The calcium values were fairly constant throughout gestation.

The total protein content was much lower in amniotic fluid than in maternal serum. In amniotic fluid it was significantly higher in the 5th month than earlier and later in pregnancy.

Small variations were noted in lactate concentration in amniotic fluid during pregnancy. It was significantly higher in the 10th month than in the 4th ($t = 3.30$). Lactate concentrations were not determined in maternal serum. However it was evident from the figures for residual anions in maternal serum (Table IV) that maternal serum lactate values were lower than those in amniotic fluid.

With the exception of the 11th to 12th weeks osmolality was significantly lower in amniotic fluid than in maternal serum. The amniotic fluid osmolality decreased towards the end of pregnancy.

Before the 33rd week the sodium/potassium ratios in amniotic fluid were not significantly different from that of maternal serum. In the 33rd to 40th weeks the values were significantly lower in amniotic fluid than in maternal serum.

The values of residual anions were much higher

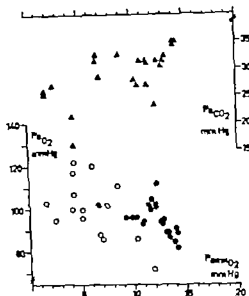


Fig. 1. Variation of amniotic fluid P_{O_2} ($P_{amn O_2}$) with maternal Pa_{O_2} (circles) and Pa_{CO_2} (triangles). Filled symbols indicate early pregnancy observations (gestational age 14–24 weeks) and open symbols late pregnancy observations (gestational age 26–39 weeks).

Table III. *Electrolyte components in maternal serum during pregnancy and in amniotic fluid at different gestational periods*

Bicarbonate values for maternal serum are standard bicarbonate values and those for amniotic fluid actual bicarbonate values. All values are given in mmol/l

	Na	K	Ca	Mg	Cl	HCO_3^-	Protein	Lactate
	Mean ± S.E.M.	Mean ± S.E.M.	Mean ± S.E.M.	Mean ± S.E.M.	Mean ± S.E.M.	Mean ± S.E.M.	Mean ± S.E.M.	Mean ± S.E.M.
Maternal serum	101 138±0.3	101 4.2 0.03	101 4.6±0.02	89 1.5±0.01	101 105±0.2	97 22.8±0.1	99 15.7±0.1	
Amniotic fluid								
Gestational weeks								
11–12	5 134±2.3	5 3.9 0.08	5 3.6±0.22	5 1.4±0.10	5 103±2.6	10 23.6±1.9		5 11.2±0.3
13–16	15 134±1.0	15 4.0±0.05	15 4.1±0.05	15 1.5±0.05	15 112±0.9	59 16.1±0.3	12 0.8±0.11	18 11.0±0.4
17–20	18 134±0.8	18 4.0 0.04	18 3.8±0.05	18 1.3±0.03	18 111±0.9	63 16.5±0.2	29 1.3±0.06	23 9.8±0.5
29–32	6 131±1.6	6 4.0±0.05	6 3.7±0.11	6 1.2±0.06	6 109±1.2	6 15.8±0.4	1 0.7	2 10.6±0.1
33–36	29 131±0.7	29 4.2±0.04	29 3.8±0.06	28 1.1±0.03	29 108±0.6	46 16.3±0.2	14 0.6±0.03	25 9.8±0.3
37–40	18 128±1.0	18 4.2±0.08	18 3.8±0.08	16 1.1±0.04	18 106±0.8	35 15.7±0.3	24 0.6±0.03	16 9.1±0.4

Table IV *Osmolality sodium/potassium ratio and residual anions in maternal serum during pregnancy and in amniotic fluid at different periods of gestation*

Values obtained after subtraction of lactate are given in parentheses

	Osmolality		Sodium potassium		Residual anions
	n	Mean ± S.E.M mosm/kg	*n*	Mean ± S.E.M mmol/l	Mean mmol/l
Maternal serum	68	284 ± 0.7	101	33 1 ± 0.2	4 4
Amniotic fluid gestational weeks					
11–12	5	281 ± 5.6	5	34.0 ± 1.3	
13–16	12	272 ± 2.8	15	33.2 ± 0.3	14.3 (3.3)
17–20	18	272 ± 1 7	18	33.9 ± 0.2	13 7 (4.9)
29–32	6	269 ± 3.0	6	33.0 ± 0.6	14.9 (4.3)
33–36	30	263 ± 1.5	29	31.2 ± 0 4	16.0 (6.2)
37–40	16	263 ± 2.3	18	30.5 ± 0.6	14.2 (5 1)

in amniotic fluid than in maternal serum. This was to a large extent due to the high lactate concentrations in amniotic fluid.

Relationship between pH and P_{CO_2}, bicarbonate sodium and lactate in amniotic fluid

The individual correlations between, on the one hand, the values of pH and, on the other hand, those of P_{CO_2}, bicarbonate, sodium and lactate were calculated in 75 samples of amniotic fluid. The correlations between pH and P_{CO_2}, and between pH and bicarbonate were highly significant ($r = -0.52$ and 0 75 respectively). The correlation between pH and sodium was significant ($r = 0.34$) and that between pH and lactate probably significant ($r = -0.24$). About the same correlation coefficients were obtained when pH was compared with the logarithms of the other parameters. A multiple regression analysis was made with pH as the dependent variable and the other variables as independent. *Highly significant correlations were found between* pH and log P_{CO_2} and between pH and log [HCO_3^-]. No significant correlations between pH and the other variables were found.

Relationship between maternal arterial blood and amniotic fluid oxygen tension and acid–base status

Mean values for P_{O_2} and acid–base status in maternal arterial blood are given in Table V The mean values show the picture of a repiratory alkalosis with metabolic compensation. The alkalosis was more pronounced in late than in early pregnancy The difference between the mean values in early and late pregnancy cases was significant for Pa_{CO_2} ($t = 3.50$) and highly significant for pH ($t = 5.80$), and probably significant for standard bicarbonate ($t = 2.59$). In different stages of gestation the differences between the mean values for Pa_{O_2} and base excess were not significant.

In early pregnancy there were no significant correlations between the values of P_{O_2}, P_{CO_2} and pH in amniotic fluid and Pa_{O_2} and acid base components in maternal arterial blood. However in late pregnancy there were probably significant correlations between P_{O_2} in amniotic fluid and Pa_{O_2}, Pa_{CO_2} and base excess in maternal blood. The correlation between P_{O_2} in amniotic fluid and Pa_{O_2} was negative

Table V *Oxygen tension and acid-base status in maternal arterial blood and amniotic fluid in a series of early pregnancies (gestational age 14–24 weeks) and in a series of late pregnancies (gestational age 26–39 weeks)*

For maternal blood standard bicarbonate values are given and for amniotic fluid actual bicarbonate values

	P_{O_2}		P_{CO_2}		pH		HCO_3^-		Base excess	
	n	Mean ± S.E.M. (mmHg)		Mean ± S.E.M. (mmHg)	*n*	Mean ± S.E.M.	*n*	Mean ± S.E.M. (mmol/l)	*n*	Mean ± S.E.M. (mmol/l)
Maternal arterial blood										
Early pregnancy	3	95.9 ± 1.2	32	31.3 ± 0.5	3	7 42 ± 0 005	32	[illegible]2.1 ± 0 2	32	−2.9 ± 0.2
Late pregnancy	17	98.5 ± 3.5	17	27 4 ± 1.2	17	7 48 ± 0.010	17	22.9 ± 0.[illegible]	17	−2 0 ± 0 4
Amniotic fluid										
Early pregnancy	32	12.4 ± 0.4	31	45.3 ± 0 4	29	7 17 ± 0 008	29	15.9 ± 0.3		
Late pregnancy	17	6.0 ± 0.8	17	51.0 ± 1.0	17	7 11 ± 0.014	17	15 7 ± 0.4		

($r = -0.55$), while the other two correlations were positive. The coefficients of correlation between amniotic fluid P_{O_2} and P_{aCO_2} was 0.51 and that of amniotic fluid P_{O_2} and maternal base excess 0.59. A multiple regression analysis between amniotic fluid P_{O_2}, maternal P_{aCO_2} and base excess gave a correlation coefficient of 0.70 when amniotic fluid P_{O_2} was the dependent variable.

DISCUSSION

The amniotic fluid P_{O_2} values found in the present investigation were very similar to those presented by Sjöstedt et al. (50, 51) but lower than values published by most other investigators. In recent methodological study by the present authors (16), it was demonstrated that errors in sampling technique, in storing of samples and in analysis of P_{O_2} easily gave too high a P_{O_2} value in amniotic fluid. It seems reasonable to suspect that technical problems of this kind may be responsible for the very high amniotic fluid P_{O_2} values found by some investigators.

In the present investigation the mean values for amniotic fluid P_{O_2} were found to be significantly higher in early than in late pregnancy. This confirms earlier findings of Sjöstedt et al. (51).

P_{CO_2} in amniotic fluid has been determined by several groups of investigators (3, 5, 10, 22, 42, 43, 44, 45, 51). The mean values in the present investigations, obtained with a P_{CO_2} electrode, were lower than those of most other investigators. This confirms the observation made by the present authors that the Astrup equilibration technique usually gave too high a value of P_{CO_2} in amniotic fluid (17). In the present investigation and in some previous reports the mean P_{CO_2} values were found to be significantly higher in late than in early pregnancy (42, 45, 51). No such difference was found by Schreiner (44).

The amniotic fluid P_{O_2} and P_{CO_2} values found in the present investigation are readily compatible with the hypothesis, originally advanced by Sjöstedt et al. (50, 51), that the P_{O_2} and P_{CO_2} of amniotic fluid tend to be related to those of foetal tissues. Such a relationship may be due to diffusion and probably also to convection through fluid movements. In that case, the lower amniotic fluid P_{O_2} values in late pregnancy may be partly explained by less effective diffusion and changed convection between foetal tissues and amniotic fluid as the foetus is growing. An additional explanation would be that the oxygen tension of foetal blood, both in the umbilical vein and artery, decreases with advancing pregnancy as previously found (26, 58). However amniotic fluid is also in contact with uterine tissue, through foetal membranes, the diffusion capacity of which is not known in vivo. No definite conclusions can therefore be made on the basis of the present results as to the quantitative origin of O_2 and CO_2 in amniotic fluid.

In early as well as in late pregnancy the mean pH values in the present investigation were of the same order as the highest mean values reported previously. Differences in the analytic procedure might be responsible for the discrepancy of lower values found by some investigators. In the present investigation, the pH values were found to be significantly lower in late than in early pregnancy. This confirms the findings of other investigators (42, 44, 45, 51). Schreiner (44) suggested that the low pH values in late pregnancy might be due to a decrease of sodium, accompanied by a lowered capacity of the bicarbonate buffer. Seeds & Hellegers (45) found increasing P_{CO_2} values and decreasing bicarbonate values with advancing gestation. Rooth et al. (42) reported increasing P_{CO_2} values, but, no significant changes in bicarbonate values with advancing pregnancy. They concluded that there was a respiratory acidosis in late pregnancy when compared with early pregnancy. The values of P_{CO_2} and bicarbonate in the present investigation confirmed the findings of Rooth et al. Also in the present investigation lower sodium values were found in late than in early pregnancy. However, in the multiple regression analysis the sodium values were not found to be of any significance for the pH values. The present investigation indicates that the difference in average pH between early and late pregnancy may be explained by differences in P_{CO_2}. Individual variations between the samples of amniotic fluid may be explained as being due to differences in P_{CO_2} as well as in bicarbonate values.

The results in the present investigation demonstrate that P_{O_2}, P_{CO_2} and pH in amniotic fluid are not usually influenced by haemolytic disease of the infant. The group of cases complicated by abnormalities other than haemolytic disease was too small and too heterogeneous to make an evaluation possible of the diagnostic value of P_{O_2}, P_{CO_2} or pH in amniotic fluid. However, the range of amniotic fluid P_{O_2} values in obviously normal cases extended down to zero. Thus, it must be concluded that the individual patient with insufficient oxygen supply

to the foetus, probably cannot be distinguished from the normal solely by the determination of amniotic fluid P_{O_2}.

The mean values for electrolyte components found in the present investigation were in agreement with previous reports on many points but differences were also noted (1 9 10 11 13 21 24 28 44 48 52, 63). Decreasing values of sodium and chloride with advancing gestation have been noted by many other investigators (9 13 28 44), although Doran et al. (11) found decreasing values of sodium but unchanged chloride values. Increasing values of potassium with gestational age were found by some investigators (11 44) but not by others (9 13). Schreiner (44) found unchanged values for calcium, in agreement with the present results. Previously reported values for lactate in amniotic fluid were lower than those in the present investigation (10 21 44). Values of total protein in the present investigation were equal to those in earlier reports (12, 38 44 57 63). Differences in amniotic fluid total protein between patients with foetuses slightly affected by haemolytic disease and those severely affected have been pointed out previously (38 57).

The mean values for osmolality in the present investigation were in agreement with those in previous reports (9 13 24 30 31), but, some have found lower values (1 34). Osmolality was not found to be a good indicator of foetal maturity in the individual case. In this respect the present results were in agreement with those of O'Leary & Feldman (34) but in contrast to those of Miles & Pearson (31).

As decreasing sodium and increasing potassium values were noted with advancing pregnancy the ratio sodium/potassium was calculated in the hope that it would give an index of foetal maturity. Although this ratio decreased with increasing gestational age, individual variations in late pregnancy were too large to permit any prognostic assumption.

In agreement with earlier reports, the acid–base status in maternal arterial blood gave the picture of a metabolically compensated respiratory alkalosis of varying degree (6, 27 36, 37 49). This has been explained by hyperventilation during pregnancy. The range of P_{aO_2} was very wide. The highest P_{aO_2} values were accompanied by the lowest P_{aCO_2} values, which indicates that some mothers may have increased their hyperventilation in the sampling situation. The lowest P_{aO_2} values may be explained by the observation of Ang et al (2), that mothers in late pregnancy had on an average a 13 mmHg lower P_{aO_2} measured on arterialized capillary blood, in the supine body position than in the sitting position.

In the present investigation a negative correlation was found between amniotic fluid P_{O_2} and maternal P_{aO_2} and a positive correlation between amniotic fluid P_{O_2} and maternal P_{aCO_2} and base excess. Other investigators found similar correlations between foetal P_{O_2} and maternal P_{aO_2} and P_{aCO_2} when moderate changes in maternal P_{aO_2} and P_{aCO_2} were induced by maternal hyperventilation (25 33). A decrease in foetal P_{O_2} was also found in induced maternal metabolic acidosis (7). The correlations between amniotic fluid P_{O_2} and maternal P_{aO_2} P_{aCO_2} and base excess may be explained by changes in foetal blood caused by varying degree of maternal hyperventilation. However influence from other parts of the uterine compartment cannot be excluded. The correlation between amniotic fluid P_{O_2} and maternal P_{aO_2} was probably dependent on variations in maternal acid-base status rather than in maternal P_{aO_2} (33).

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FETO-MATERNAL TRANSFUSION AND FREE BLEEDING FROM THE UMBILICAL CORD

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Abstract. The incidence of feto-maternal transfusion in 200 normal women as determined from blood samples taken hen labour had become established, with contractions occurring approximately every 5 minutes, and again 30 minutes after delivery

In 90 instances, after cessation of umbilical artery pulsation, the umbilical cord was ligated and cut, allowing free flow from the placenta. In the remaining 110 instances the cord was cut between two pairs of forceps.

No difference in feto-maternal transfusion in the two groups could be demonstrated.

In the later months of pregnancy fetal erythrocytes, indicati of transplacental bleeding, can frequently be demonstrated in the mother's cir culation. During the birth process, both the frequency and quantity of this transfusion increases (Kleihauer & Betke, 1960· Woodrow Clarke, Donohoe, Finn, McConnell, Sheppard, Lehane, Russell, Kulke & Durkin, 1965 Sullivan & Jennings, 1966)

About 1960 prophylactic treatment of the mother with anti-D-immunoglobin was instituted after delivery when there was rhesus incompatibility This has been routine procedure in Denmark since 1969 (Andersen, Freiesleben & Lundsgaard, 1969). As in other countries, the standard dose given is 300 µg anti-D-immunoglobin. This is effectiv protection against immunization in most cases, but when there is a transfusion of larger quantities of fetal blood, the dose can be inadequate (de Wit & Borst Eilers, 1968 Woodrow Bowley Gilliver & Strong, 1968).

Supplies of anti-D-immunoglobin are limited, and therefore any procedure which can reduce the incidence of feto-maternal transfusion is of therapeutic interest. In 1970, Terry demonstrated that cutting of the cord immediately after birth, and establishment of free flow out from the placenta could reduce the quantity of feto-maternal transfusion.

In Denmark, cutting of the cord is generally delayed until cessation of pulsation in the umbilical artery This study was designed to evaluate establishment of free flow out from the placenta after this delay

MATERIAL

200 normal women f comparable age and parity in labour were studied. Mothers and children's ABO-groups were compatible.

On admission to the department, the women were generally in labour with intensifying contractions at approximately 5 min intervals. 5 ml venous blood was withdrawn into heparin glass (test I). Thirty minutes after birth, the venous puncture was repeated (test II).

The control group comprised 110 mothers born on odd dates. On delivery of the leading shoulder, 0 2 mg methyl-ergometrine (Methergin®) was given intramuscularly On cessation of cord pulsation, the cord was cut between two pairs of forceps. Placental delivery was spontaneous.

The study group comprised 90 mothers, born on even dates. Birth was conducted as in the control group. On cessation of cord pulsation, the cord was cut, allowing free flow out from the placenta.

METHOD

Fetal erythrocytes were demonstrated as described by Kleihauer & Betke (1960) A thin even smear was fixed with 80% ethanol for 5 min. Hb A was extracted with citric acid phosphate buffer pH 3.2, at 37°C for 5 min. After haematoxylin and eosin staining, the maternal erythrocytes appeared as ale red shadows, hile the Hb-F containing fetal erythrocytes are deep blue-red.

The maternal erythrocytes in 5 fields, and the fetal erythrocytes in 50 fields were counted (40 enlargement).

Table I *Feto-maternal transfusion during delivery when the umbilical cord is clamped after the cessation of cord pulsation*

Sample I Blood taken before deli ery
Sample II Blood taken 30 min after delivery

	Feto-maternal transfusion, ml								
Control group	0	0–0.5	0.5–1	1 2	2–4	>4	Total	$\bar{x}$	s
Sample I									
No of patients	80	0	10	11	4	5	110	0.71	1 64
Percent	72.7	0	9 1	10.0	3 6	4.5			
Sample II									
No. of patients	76	2	8	10	4	10	110	0.91	2 08
Percent	69 1	1.2	7.3	9 1	3.6	9 1			

The amount of feto-maternal transfusion was calculated by the method of de Wit & Borst Eilers (1969).

RESULTS

In the control group fetomaternal transfusion was noted in 27.3% Mean transfusion was 0 71 ml, S D 1 64 ml (Table I) In the study group there was transfusion in 25 6% Mean transfusion was 0.50 ml, S D 1 05 ml (Table II) At this stage, therefore the two groups were comparable ($t = 0.90$ and $0.20 < p < 0\ 40$)

Thirty minutes after delivery fetomaternal transfusion was found in 30.9% of control group mothers (mean 0.91 ml, S D 2.08 ml Table I) and in 31 1% of the test group (mean 0 67 ml, S D 1 65 ml Table II) The difference is not statistically significant ($t = 1\ 18$ and $0.20 < p < 0\ 40$)

Feto-maternal transfusion occurred in the interval between tests in 15 control group and 12 test mothers (Table III) $\chi^2 = 0.09$ No difference is thus apparent in the number of mothers in whom feto-maternal transfusion occurred during the birth process itself

The amount of the fetomaternal transfusion in tests I and II was of the same order in both groups ($0.40 < p < 0\ 60$)

DISCUSSION

Many factors influence feto-maternal transfusion. Hellman, Tricomi & Gupta (1957) demonstrated pressures higher in the villous vessels than in the inter villous spaces unintended lesions of the placenta during amniocentesis can predispose to the passage of fetal cells to the mother (Zipursky Pollock, Chown & Israels, 1963) and obstetrical intervention e.g Caesarean section and manual removal of a retained placenta, likewise increases the risk of feto-maternal transfusion (Zipursky Pollock, Neelands, Chown & Israels, 1963).

Table II *Feto-maternal transfusion during del very when free bleeding from the umbilical cord was established after the cessation of cord pulsation*

Sample I Blood taken before deli ery
Sample II Blood taken 30 minutes after delivery

	Feto-maternal transfusion, ml								
Experimental group	0	0–0.5	0.5–1	1 2	2–4	>4	Total	$\bar{x}$	s
Sample I									
No of patients	67	3	2	10	5	3	90	0.50	1.05
Percent	74 4	3.3	2.2	11 1	5 6	3 3			
Sample II									
No. of patients	62	3	4	12	7	2	90	0.67	1 65
Percent	68 9	3.3	4 4	13 3	7.8	2.2			

Table III *Number of patients in whom no feto–maternal transfusion was detected and number of patients in whom transfusion occurred between the two blood tests*

	No fetal blood in sample II	With fetal blood in sample II	Total
Experimental group	55	12	67
Controls	65	15	80

Post partum, the uterus contains from ~20% fetal erythrocytes (Queenan, Landesman, Nakamoto & Wilson, 1962), and labelled erythrocytes instilled, post partum, into the uterus can subsequently be detected in the mother's circulation (Queenan et al., 1962).

If the umbilical cord is cut immediately after delivery of the child, and free flow of blood out from the placenta allowed, the number of mothers with feto-maternal transfusion is reduced, as is also the amount of the transfusion when it does occur (Terry 1970). This probably results partly from reduced pressure in the fetal villous vessels, and partly from reduction in the post-partum uterine content of fetal blood cells.

Danish practice is to preserve the cord until the cessation of umbilical arterial pulsation. This allows the free passage of blood from placenta to child. In this study the cord was not cut until after the cessation of umbilical arterial pulsation, and it was found that, at this stage, the incidence and amount of feto-maternal transfusion was not reduced.

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FETAL HEART RATE VARIATION DURING UNCOMPLICATED LABOUR

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Abstract. A prospective longitudinal and cross-sectional study of FHR was performed on 110 healthy multiparae during normal labour. Auscultations during 15 second-periods were performed immediately after a contraction and 1 min later and related to the station of the fetal head. No significant differences in mean-FHR were found between related early and late observations during the whole process of labour.

The mean-FHR decreased significantly during the passage of the fetus through the birth canal. During the second stage the cross-sectional biological variation of FHR was twice as large as during the first stage. In the individual subject the maximal difference between related early and late observations following a contraction increased with 70% when the fetal head reached the pelvic floor.

The data are compared with those from a previous investigation on nulliparae. Auscultograms for practical clinical use are presented.

Electronic recordings have contributed to a detailed knowledge of fetal heart rate (FHR) variations in relation to uterine contractions during labour (Caldeyro-Barcia et al., 1966; Hon, 1960, 1966; Mendez-Bauer et al., 1963).

A recent investigation (Huldt & Westin, 1969) showed a decreasing frequency and an increasing biological variation of the fetal heart rate in nulliparae during normal labour (Fig. 1). The results were based on a prospective longitudinal study with several auscultatory observations in intervals between contractions. A careful methodological analysis showed the maximal auscultation error to be 3.5%, that is about 1 beat/15 sec auscultation period.

The proven dynamic changes of FHR during labour in nulliparae made it important to extend our investigation also to multiparae.

MATERIAL AND METHODS

The study was conducted at the Obstetric Department of Sabbatsberg Hospital. 110 healthy women who had previously borne at least one child were examined during normal labour (Table I). FHR was determined by auscultation with a wooden stethoscope 0–15 and 85–100 sec after uterine contractions (Fig. 2). A total of 986 FHR observations were obtained. On each occasion the fraction of the head above the pelvic inlet, the diameter of the cervix and the station of the head were recorded. In addition, 9 healthy multiparae were auscultated in duplicate 1:1 ... pre-term.

RESULTS

The mean FHR before onset of labour was 33.7 ± 0.3 (S.E.) beats/15 sec. At the onset of labour it rose to 35.4 ± 0.4 ($p < 0.01$).

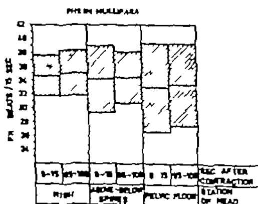
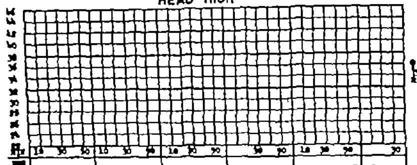
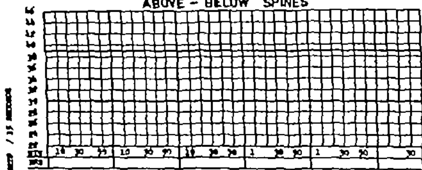
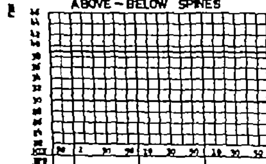


Fig. 1. FHR during normal nulliparous labour. Mean values and 95% confidence limits in relation to different stations of fetal head (Huldt & Westin, 1969). FHR for above, at and below spines have been combined (mid-pelvic station).







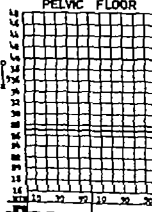


Fig. 5. Auscultogram for nulliparous labour. Constructed for routine clinical use. Cross-sectional 95% confidence limits as well as maximal difference in the individual subject between auscultations 0–15 and 85–100 sec after uterine contraction.

Table I *Our criteria of normal delivery*

Mother	
Gestational period 38–42 weeks	
Duration of labour less than 12 hours	
No operative termination of deli ery	
Child	
No passage of meconium during labour	
No umbilical cord complication	
Occiput anterior presentation	
Birth weight at least 2 500 grams	
Spontaneous respiration within 1 minute	
Apgar score 8–10 at 1 minute	

Fig. 3 illustrated the mean values and the 95% confidence limits of FHR during normal labour There is a significant decrease ($p < 0.01$) of the mean FHR when the head descends from a posi tion high in the pelvis to the mid-pelvic station During the passage through the mid pelvis there are no such changes. When the head descends to the pelvic floor the mean FHR again decreases significantly ($p < 0.001$) At each station of the fetal head there is no difference in mean-FHR between observations performed 0–15 and 85–100 sec after contraction. For the stations high, mid-pelvis, and pelvic floor the mean FHR are respectively 35.4 34.4 and 32.4 beats/15 sec (Fig. 4) The limits are given with 95 con-fidence and indicate an increasing biological variation of FHR with descent of the fetal head through the birth canal.

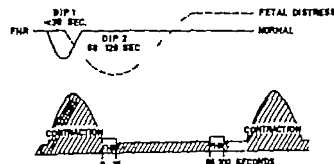


Fig FHR was auscultated 0–15 and 85–100 sec afte uterine contractions. The end f contraction was de termined by palpation.

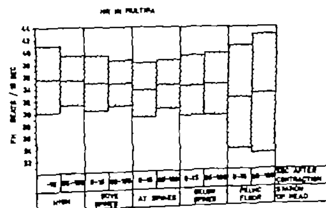


Fig 3 FHR during normal multiparous labour Aus-cultations were performed 0–15 and 85–100 sec after uterine contractions and related to station of fetal head.

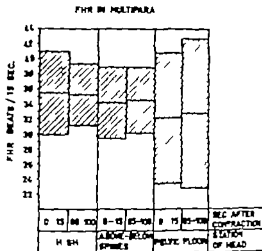


Fig 4 FHR in multiparous labour. Mean alues and 95% confidence limits in relation to different stations of fetal head (compare with Fig. 1).

By longitudinal statistics the maximal dif ference between FHR 0–15 and 85–100 sec in the individual subject was calculated and is shown in Table II together with corresponding data from the nullipara-investigation.

Comparison between FHR in nulli and multiparous labour

At each station of the fetal head there is no dif ference between the mean FHR in nulliparae and multiparae during normal labour (Figs. 1 and 4)

Table II *Maximal FHR differences (4 S D) beats/15 sec for the individual subject*

Station of fetal head	0–15 VS 85–100 sec within same interval: Nullipara	Multipara
High	3	7
Mid-pelvis	5	7
Pelvic floor	7	12

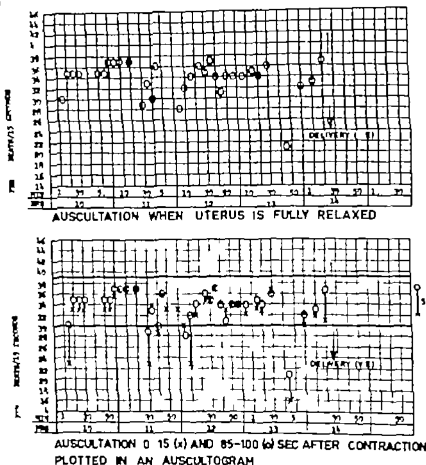


Fig. 7. Conventional auscultation versus the auscultogram method. 25-year-old term nullipara. Mid pelvis station. Child 3 250 g, cord around the neck. Delivery by vacuum extraction. Apgar score 3 at 1 min and 8 at 5 min. The auscultogram method revealed signs of impending fetal distress 3 / hours earlier than conventional auscultation.

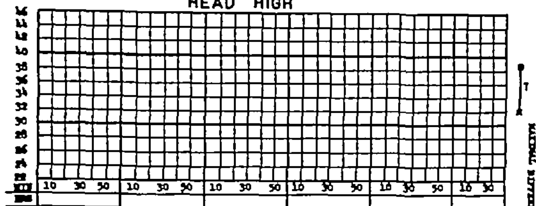


Fig 6 Auscultogram for multiparous labour Constructed for routine clinical use. Cross-sectional 95 confidence limits as well as maximal difference in the individual subject between auscultations 0–15 and 85–100 sec after uterine contraction.

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PREGNANCIES DURING ORAL CONTRACEPTIVE TREATMENT

Swedish Experiences

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Abstract At the beginning of 1969 Swedish physicians were called upon by the National Board of Health and Welfare to report all pregnancies known to have occurred in women who had used oral contraceptives. Ninety pregnancies were reported for 1968, 73 of these with the sequential type of therapy and 17 with the combined type. Calculations using Pearl's formula and based on sales figures gave pregnancy rates of 0.467 and 0.005 for sequential and combined regimes respectively. The possible causes of errors in these statistics are discussed. However concluded that the pregnancy risk is much more than ten times higher with the sequential than with the combined type of therapy. The most important cause of the failures in this series seems to have been the lack of efficacy of the drugs.

The first oral contraceptives, which were introduced through Pincus' investigations in Puerto-Rico 1955–56 contained comparatively high amounts of oestrogenic and progestogenic components. These drugs were accepted for sale in USA in 1960. These combined preparations showed a good contraceptive effect as well as various types of side-effects. Great efforts were made to develop preparations with less side-effects by reducing the hormonal content to degrees which would not at the same time lessen the contraceptive effect. The main results were the introduction of combined preparations with lower hormonal content and sequential preparations. After extensive clinical trials by Goldzieher among others, the first sequential preparation was accepted in USA in 1965.

The usual estimation of the efficacy of contraceptives is made according to Pearl's formula which shows the pregnancy rate per 100 woman years with 12 cycles per year. This rate has been estimated at between 0 and 2.7 for the oral contraceptives (5). It has been difficult to establish differences in the efficacy of the preparations as the results vary considerably in different investigations. In particular the difference in safety between combined and sequential preparations has been questioned. However after an extensive study of the literature, Hines & Goldzieher in USA (2) did not find such a difference. The Swedish authorities first sanctioned the use of a combined preparation for contraceptive purposes in 1964. The first sequential preparation was registered in 1966. During 1967 and 1968 increasing numbers of reports of pregnancies during oral contraceptive treatment, nearly all with sequential preparations were received by the National Pharmaceutical Laboratory (Statens farmacevtiska laboratorium SFL). At this time there were 16 combined and two sequential preparations on the market. A third sequential preparation was marketed in the autumn of 1968. Judging by the reports the difference in efficacy between the two types of preparation seemed to be considerably larger than had been stated earlier. Among Swedish gynaecologists who were contacted by the pharmacotherapeutic department of the SFL, some considered it evident that the sequential preparations provided less effective contraceptive protection while others considered the difference to be very small if the tablets were taken regularly. During 1968 reports came from Britain that a considerably higher pregnancy rate for the sequential preparations had been found there too. Owing to the importance of the question and the divergent opinions in Sweden as well as in other countries, a closer investigation was considered justified.

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In multiparous labour when the head is high in the pelvis and at the pelvic floor the cross-sectional variation is significantly larger For the individual subject the biological variation is significantly larger for the multiparae during the entire labour (Table II)

DISCUSSION

The increased mean FHR at the onset of labour suggests an increase in fetal sympathetic tone and is possibly an expression of maternal stress, a factor which may also be important in relation to the wide FHR variations during the second stage of labour

When the head is compressed during its passage through the mid-pelvis vagal stimulation causes a slight decrease in mean FHR. This assumption gains further support from the marked decrease of the mean FHR when the head reaches the pelvic floor and is further compressed by the bearing down efforts of the mother The great variations of FHR during the second stage indicate that for the fetus this is probably the most critical part of the whole passage through the birth canal and should not be prolonged. Considering the normal cross-sectional and longitudinal limits, intelligent auscultation immediately after a contraction and 1 min later has proved to be a sensitive method for the early detection of fetal distress (Huldt & Westin, 1969) The auscultograms for nulliparae (Fig 5) and for multiparae (Fig. 6) are constructed for routine clinical use

Fig. 7 compares conventional auscultation during the uterine relaxation periods with auscultation 0–15 and 85–100 sec after uterine contractions. A 25-year-old nullipara was, by a mid-pelvic vacuum extraction, delivered of an asphyxiated child with the umbilical cord around his neck. Conventional auscultation disclosed a pathological dip of FHR 40 min prior to delivery but auscultation according to our method revealed signs of impending fetal distress three-and-a-half hours earlier

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Table II. *Oral contraceptives registered on the Swedish market in 1968 and pregnancies reported this year during such treatment*

	Progestagen	Oestrogen	Number of pregnancies	Estimated number of cycles during treatment	Pregnancy rate acc. to Pearl's formula
Combined preparations					
Lynestrenol	5 mg +	M 0.15 mg			
Lyndiol			0	3 511	—
Megestrol acetate	2 mg +	M 0.1 mg			
Delpregnin			1	157 118	0.0076
Chlormadinone acetate	3 mg +	M 0.1 mg			
Aconcen			0	54 720	—
Norethynodrel	2.5 mg +	M 0.1 mg			
Previson			0	6 684	—
Norethisterone	5 mg +	M 0.1 mg			
Conlumen			1	343 893	0.0035
Ortho-Novin			0	36 972	—
Norethisterone	1 mg +	M 0.1 mg			
Conlunett			6	1 161 180	0.0062
Ethynodiol diacetate	1 mg	M 0.1 mg			
Ovulen			0	287 598	—
Norethisterone	1 mg	M 0.08 mg			
Orthonovin mite			0	12 466	—
Norethynodrel	5 mg	M 0.075 mg			
Enavid mite			0	1 347	—
Lynestrenol	2.5 mg	M 0.075 mg			
Lyndiol mite			6	898 003	0.008
Noracyclin			0	22 185	—
Medroxyprogesterone acetate	10 mg +	EO 0.05 mg			
Provest			1	39 331	0.031
Norethisterone acetate	4 mg	EO 0.05 mg			
Anovlar			0	107 116	—
Norethisterone acetate	3 mg	EO 0.05 mg			
Anovlar mite			2	721 747	0.003
Norgestrel	0.5 mg	EO 0.05 mg			
Follinyl			0	12 766	—
Total			17	3 867 337	0.005
Sequential preparations					
Chlormadinone acetate	2 mg	M 0.08 mg			
Sequens			33	62 343	0.635
Megestrol acetate	1 mg	EO 0.1 mg			
Ovanon			40	121 043	0.396
Non ovlon			0	3 953	—
Total			73	187 341	0.467

M Mestranol EO Ethinyloestradiol

the relationship between pregnancies occurring during the first cycles of treatment and the others there seems to be no significant difference between the two groups. However somewhat more pregnancies seem to have occurred during the first 5 months of treatment with the sequential preparations than with the combined preparations.

With the aid of the information volunteered by the women an attempt has been made to attribute the causes of the pregnancies to tablet failure (deficient effect) or patient failure. Among patient failures are included forgotten tablet, interval longer than 7 days between the treatment cycles (which occurred particularly with the preparation Sequens), vomiting and diarrhoea during the cycle in question or in one case, the fact that the patient thought herself to be pregnant before the beginning of the treatment. Cases were considered to be tablet failures when the woman stated with certainty that she had taken the

Table I *Total pregnancies by years*

Year of pregnancy	Number of pregnancies during sequential treatment	Number of pregnancies during combined treatment	Total
Unknown	2		2
1964		2	2
1965	1	2	3
1966	3	3	6
1967	39	4	43
1968	73	17	90
1969	10	10	20
Total	128	38	166

METHODS

Reliable data about all pregnancies with the two types of oral contraceptives were not available from sufficiently large Swedish population, and for various reasons they could not be obtained.

Instead, at the beginning of 1969 a circular was issued from the National Board of Health and Welfare (Socialstyrelsen) to all physicians in Sweden. They were requested to report to the pharmacotherapeutic department all pregnancies occurring during oral contraceptive treatment which had been observed before this time. They were further asked to state the date of birth of the patients, any previous pregnancies, type of cycle, name of preparation, length of treatment, if the preparation had been taken in accordance with the instructions, etc. As the pharmacotherapeutic department considered a uniform interrogation of all the women valuable, attempts were made also by the department to reach them by telephone.

RESULTS

In reply to the circular and through other reports, partly via the Adverse Drug Reactions Committee, 166 pregnancies which had occurred from 1964 to 1969 during oral contraceptive treatment were reported to the pharmacotherapeutic department.

The distribution of the pregnancies is shown in Table I. The largest number of reported pregnancies occurred in 1968. This can partly be explained by the steadily increasing use of oral contraceptives and partly by the introduction of a new sequential preparation towards the end of 1967. The reports from the earlier years tend to be less complete because of the time which had elapsed before the reports were requested. The year 1969 was not intended to be included in the investigation and the reports for this year principally relate to the beginning of the year. For these reasons the analysis of the material was concentrated on the year 1968.

During 1968 90 pregnancies were reported. 73 of these were in women treated with sequential preparations and the other 17 in women who had used combined preparations. For five of the pregnancies no information could be obtained except date of birth, time of pregnancy and type of preparation. For the remaining 85 cases data have been obtained to the extent which is shown in the tables. In 71 of them personal questioning was possible. Through the courtesy of the AB Läkemedelsstatistik (Drug Statistics Ltd) the sales figures for these preparations during 1968 have been obtained and from these figures the number of treatment cycles for the different preparations as well as the pregnancy rate according to Pearl's formula were calculated.

For a correct estimation of the pregnancy rate with this formula, pregnancies due to patient failure (e.g. tablet forgotten) as well as tablet failure (deficient effect) should, according to Tietze (5) be included, and so no differentiation of the causes of the pregnancies has been made.

The results are shown in Table II. It is evident that the pregnancy rate figures lie fairly close to each other for the different combined preparations as well as for the different sequential preparations, while the difference between the two groups is large. The combined preparations dominated the market markedly during 1968 with nearly 4 million treatment cycles as compared with about 190 000 for the sequential preparations, while the relations were the opposite for the occurrence of pregnancies. According to Pearl's formula the pregnancy rate for combined preparations is 0.005 and for sequential preparations, 0.467. The results indicate a hundredfold greater risk of pregnancy with the use of sequential preparations than with the combined ones.

A closer examination of the material has been made to assess whether there are any essential differences in age distribution or fertility between the two treatment groups (Tables III and IV). However the results show a fairly good correspondence between the two groups with regard to age distribution and previous pregnancies.

In Table V the treatment c[illegible] pregnancies occurred are show[illegible]

have been taken for the purpose of contraception. The sale during the later part of 1967 was probably not considerably different from that during 1968 and for this reason a potential shift between sale and consumption has been judged to play an insignificant role. Combined preparations are also used in the treatment of gynaecological disturbances, but the preparations are then usually of another potency. In any case they constitute a very small proportion of the total consumption. It is very likely that the tablets sold to a very large extent really ha e been taken by the women. We have estimated that these causes of error taken together at the very most reduce the number of treatment cycles by 5%.

The reporting of pregnancies may be incomplete principally because of forgetfulness. Since the reports in the lay press have concerned the sequential preparations in particular it is possible that these pregnancies have been reported to a comparatively large extent, while certain pregnancies during combined treatment have been forgotten. This view tends to be confirmed by the fact that the pregnancy rate for the combined preparations is rather low compared with data from the literature. However the latter is in most cases derived from limited, controlled, clinical trials of different preparations.

The figures now presented are closer to those which have been obtained from a comparison between reported pregnancies and the sales figures for a preparation published by the Committee on Safety of Drugs in 1969 where the sequential preparations turned out to be ten times less safe than the combined preparations. In the present series the efficacy of the sequential preparations is considerably less than that of the combined and according to the estimates about hundred times lower. E en if the causes of error discussed above are considered it seems obvious that the sequential preparations are much more than ten times less safe than the combined preparations.

E en when the sequential preparations are included the protection against pregnancy is still much better than with other non-oral, contraceptives. For those women who cannot take combined oral contraceptives owing to subjective side-effects the sequential method is, thus, a reasonable alternative. However the information concerning its lesser safety must be complete.

The number of pregnancies in the higher age groups is remarkable when it is considered that the consumption of oral contraceptives is proportionately greater in lower age groups. However the present series consists almost entirely of married fertile women who, perhaps, have not had so strong a motivation and have subconsciously fallen victims to forgetfulness.

When studying the causes of the pregnancies it is evident that with sequential treatment there is a considerable predominance of tablet failure over patient failure. This agrees with the fact that the sequential preparations are less safe than the combined.

Concerning different causes of patient failure the figures are too small to allow general conclusions. Forgetfulness has been stated by proportionately more women in the combined group and with Sequens prolonged intervals between the treatment cycles seems to have played a role. It has not been possible to erify essential differences in these respects between sequential and combined preparations.

Comparison between the two sequential preparations Ovisec and Sequens shows that the pregnancy rate with Ovisec is inconsiderably lower than that with Sequens in spite of the "safer" administration of Ovisec (with 7 placebo tablets) and in spite of the fact that Ovisec contains a proportionately higher dose of oestrogen (0.1 mg ethinyloestradiol).

Although there have been reports of masculinisation of the fetus following large doses of progestogens to the mother there has been no report of such abnormal development after oral contraceptive dosage during early pregnancy. Nor did any of the 45 children in this series born before the in estigation was ended, show any abnormal development of the external genitalia, even though the mothers had taken their contraceptive pills from 1 to 5 months during early pregnancy. One of the children was born with six fingers, one with congenital dislocation of the hip and one with phenylketonuria, but there is no evidence that these abnormalities are due to the hormonal steroids. There were nine spontaneous abortions.

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Table III *Age distribution of women who became pregnant during oral contraceptive treatment 1968*

Age, years	Sequential	Combined
Unknown		2
<20	2	2
21–25	13	3
26–30	12	4
31–35	28	5
36–40	14	0
>40	2	1
Total	73	17

Table IV *Fertility before treatment 1968*

Number of pregnancies	Sequential	Combined
Unknown	6	3
0	8	1
1–2	28	8
3–4	4	3
>4	7	2
Total	73	17

Table V *Pregnancy related to number of cycle during treatment 1968*

Cycle	Sequential	Combined
Unknown	2	4
I	6	1
II–III	12	1
IV–V	10	2
>V	33	9
Total	73	17

tablets in accordance with the instructions and there were no other noteworthy circumstances. The results of the available data are shown in Table VI which demonstrates a significant predominance for deficient effect in the sequential group.

In an attempt to obtain a better picture of the probable causes of the pregnancies in a somewhat larger series an analysis has been made of the whole material from 1964–69 in Table VII. These data are presented with reservations for the reasons mentioned in the discussion. The women who admitted forgetfulness constitute a fifth of the whole number in the combined group and a tenth of the sequential group. As to vomiting and diarrhoea, these symptoms were of so serious a nature in 5 cases in the combined group that they were considered as a very probable cause while they could be considered as a probable cause in only one case in the sequential group. Short and irregular cycles have been mentioned as an uncertain factor. Eight women in the sequential group and three in the combined group had irregular and/or short cycles. Mears (3, 4) and Borell (1) consider that an immediate change from a "stronger" hormone preparation to a "weaker" involves an increased risk of pregnancy during the first months after the change. In the whole series 19 of 52 women in the sequential group who became pregnant in the first to third treatment cycle had previously been treated with a combined preparation and two of seven in the combined group had previously taken a stronger combined preparation. However about two-thirds of all pregnancies occurred in later cycles.

DISCUSSION

When estimating the number of treatment cycles we have assumed that all tablets sold during 1968

Table VI *Distribution of tablet failures, patient failures 1968*

Cause	Sequential	Combined
Unknown	10	2
Tablet failure	53	8
Patient failure	10	7
Total	73	17

Table VII. *Distribution tablet failures patient failures 1964–69*

Cause	Sequential	Combined
Unknown	26	7
Tablet failure	72	15
Patient failure		
Tablet forgotten	3	4
Tablet forgotten, taken next day	7	
More than 7 day interval between treatment cycles (Sequents)	11	—
Vomiting or diarrhoe	1	5
Short and or irregular cycles	8	3
Pregnant before commencement of treatment		2
Total	128	38

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OXYGEN TENSION, CARBON DIOXIDE TENSION AND pH IN AMNIOTIC FLUID AND MATERNAL ARTERIAL BLOOD DURING INDUCED MATERNAL HYPEROXIA AND HYPOXIA

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Abstract. The effect of maternal hyperoxia and hypoxia on P_{O_2}, P_{CO_2} and pH in maternal arterial blood and in amniotic fluid was studied in 8 pregnant women in the second trimester. Hyperoxia with about 100 % O_2 gave an increase in maternal P_{aO_2} to about 500–600 mmHg followed by a significant increase in amniotic fluid P_{O_2}. Pronounced hypoxia (10 % O_2) reduced maternal P_{aO_2} to about 40 mmHg and caused a significant decrease in amniotic fluid P_{O_2}. Minor changes in P_{CO_2} and pH of maternal arterial blood and amniotic fluid were seen during hyperoxia and hypoxia.

INTRODUCTION

Using a technique described by Saling (20), oxygen tension and acid-base status can be measured in foetal scalp blood during delivery. It has not been possible to make direct measurements of these parameters in human foetal blood before the onset of labour and rupture of membranes.

Starting with the pioneer works of Sjöstedt, Rooth and Caligara (17, 24, 25) several studies of P_{O_2}, P_{CO_2} and pH in amniotic fluid have been made with the hypothesis that such values might reflect the state of oxygenation of the foetus. In a recent report by the present authors (9) it was concluded that the observed values of P_{O_2} and P_{CO_2} were compatible with the original hypothesis of Sjöstedt et al. However, in other reports the P_{O_2} values in the amniotic fluid were considerably higher and thus incompatible with the hypothesis (14, 15, 27). It is therefore obvious that the relationship between the oxygen and carbon dioxide tensions in amniotic fluid and the state of oxygenation of the foetus still needs further clarification.

Earlier studies of the effect of maternal hyperoxia on amniotic fluid P_{O_2} gave inconclusive results. Vasicka et al. (28) found a rise in amniotic fluid P_{O_2} after 5 min of maternal inhalation of 100 % oxygen. These findings were confirmed by Romney et al. who exposed mothers to hyperoxia with 100 % oxygen or to general anaesthesia with 50 % oxygen and 50 % cyclopropane (16). In a later publication Vasicka & Hutchinson (27), using an intra-uterine tissue electrode, were unable to demonstrate significant changes in amniotic fluid P_{O_2} after 13–16 min of maternal inhalation of 100 % oxygen. Bertolizio et al. (3) found no significant changes in amniotic fluid P_{O_2}, P_{CO_2} or pH after 45 min of maternal hyperoxia with 100 % oxygen. Schreiner (21) found, in most cases, that maternal hyperventilation with ambient air gave a decrease of amniotic fluid P_{CO_2} while hyperoxia with 100 % oxygen gave no systematic changes of P_{CO_2} or pH in amniotic fluid. Studies of the effects of maternal hypoxia on amniotic fluid P_{O_2}, P_{CO_2} and pH have not, to our knowledge, been reported in the literature.

In the present publication, the effects of induced maternal hyperoxia and hypoxia on amniotic fluid P_{O_2}, P_{CO_2} and pH and on maternal arterial blood P_{O_2} and acid-base status are discussed.

SUBJECTS AND PROCEDURES

The investigations were performed on 8 pregnant women admitted to the hospital for legal abortion. The duration of pregnancy varied between 15 and 20 weeks, calculated from the first day of the last menstrual period.

The procedure and its objective was described to the mothers. They were expressively invited to participate and given a free choice. Thus, free and informed consent had been given in all cases. There were no complications resulting from the procedure.

The patients were examined in the supine position. The amniotic fluid samples were drawn into siliconized all-glass

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Table I. *Oxygen tension and acid-base status in maternal arterial blood and amniotic fluid during normoxia, hyperoxia and hypoxia, caused by variations of the O_2 fraction of inspired gas* (F_{IO_2})

Time is given in minutes from the start of each experiment

Case no	F_{IO_2}	Time (min)	Maternal arterial blood Po_2 (mmHg)	Pco_2 (mmHg)	pH	$[HCO_3^-]$ mmol/l	Amniotic fluid Po_2 (mmHg)	Pco_2 (mmHg)	pH
1	21	0	85	31.5	7.43	22.5	13.9	43.9	7.20
	100	15	484	29.0	7.45	21.5	22.1	43.6	7.20
	21	25	86	30.5	7.42	21.5	22.0	43.6	7.20
	15	35	60	29.5	7.43	21.5	16.3	42.9	7.20
	10	45	39	24.0	7.48	21.5	13.7	42.2	7.21
	21	45	80	32.5	7.42	21.5			
	21	63					13.4	42.3	7.21
2	21	0	94	33.0	7.39	21.0	11.0	46.5	7.10
	100	18					31.0	47.1	7.08
	21	30					24.4	45.9	7.08
	21	45					20.8	46.4	
	21	60					15.6	46.4	
	21	75					15.8	48.0	
	21	90					15.1	47.6	7.07
3	21	0					14.5	48.5	7.11
	15	10					13.1	45.0	
	10	20					8.2	47.3	7.11
	21	40					12.7	47.9	
4	21	0					14.4		
	15	10					11.6		
	10	20					9.3		
5	21	0	99	33.0	7.41	22.0	14.6	46.5	7.20
	10	10	42	23.5	7.53	23.5	12.3	47.3	7.21
	21	35					15.3		
	100	90	628	30.0	7.44	22.5	20.8	40.5	7.19
	21	[illegible]					14.3		
6	21	0	91	34.5	7.41	23.0	14.1	42.5	7.19
	15	10	41	32.5	7.42	22.5	14	42.3	7.19
	10	15	43	30.5	7.46	23.5	10.9		
	10	20	41	30.0	7.46	23.5	11.0	41.6	7.20
	21	40					13.5		
	100	55	604	30.5	7.42	21.5	15.9	42.0	7.19
	21	85					11.6	42.2	7.18
7	21	0	85	29.5	7.42	21.5	13.9	47.1	7.17
	100	15	604	29.5	7.41	20.5	17.2	47.0	7.16
	100	30	602	30.0	7.41	20.5	23.7	45.6	7.13
	100	45	576	31.5	7.40	21.0	23.7	46.2	7.13
	100	60	597	30.5	7.41	21.0	24.1	45.9	7.12
	21	90					12.0	47.5	7.14
	21	120					11.9	47.5	7.12
8	21	0	94	33.5	7.42	22.5	12.6	49.7	7.11
	100	15	505	30.0	7.46	22.5	17.7	48.9	7.10
	100	30	483	29.5	7.46	22.5	21.3	46.7	7.11
	100	45	491	30.0	7.45	22.5	20.5	46.2	7.10
	100	55	599	37.0	7.37	21.5	20.2	46.9	7.12
	21	85					13.3	46.9	7.12
	21	115					12.4	47.1	7.11

In case 8 the experiment was interrupted at time 55 min as the exhalation valve outlet was obstructed.

syringes through an indwelling catheter with an outer diameter of 1.2 mm. The catheter was introduced by transabdominal puncture of the amniotic cavity under local anaesthesia. Samples of maternal arterial blood were drawn into heparinized and siliconized all-glass syringes through a polyethylene catheter introduced into the brachial or radial artery All syringes with amniotic fluid or arterial blood samples were immediately sealed with a metal cap and stored in iced water until analyses were performed.

Hyperoxia was induced with about 100% oxygen and hypoxia with about 15% oxygen in nitrogen or 10 % oxygen in nitrogen. The mothers breathed the gas mixtures through an open system val with a rubber mouth piece using a nose-clip. The gas mixtures were delivered from Douglas bags or directly from gas tubes with a pressure-reducing rubber bag attached to the system.

ANALYTIC METHODS

Analyses of P_{O_2}, P_{CO_2} and pH in amniotic fluid were made with Radiometer electrodes (E 5046, E 5036 and G 297/G 2) at 37 0±0 1 C. These analyses were performed using the same technique as described earlier (7 8) The combined errors of sampling and analysis gave the following limits of significant differences at the 95% confidence level for the single value fo P ±1.5 mmHg, for P_{CO_2} ±2.3 mmHg and for pH±0.02.

Analyses of P_{O_2} and pH in maternal blood were made with the same equipment as for amniotic fluid. The P_{O_2} lectrode was calibrated by using gas mi tures with about the same P_{O_2} lev ls as expected in the samples. P_{CO_2} in maternal ar terial blood in the first 2 cases was determined by the Astrup equilibration technique (22, 23), and standard bicarbonate and base excess calculated from a Siggaard-Andersen nomogram. In the remaining 6 cases, maternal arterial Pco was analysed with P_{CO_2} electrode, and standard bicarbonate and base excess were calculated with a Blood Gas Calculator (Type BGC 1 Radiometer) The random error of a single determination in blood was ( ) for P_{O_2} 0 7 mmHg t a mean alue of 77 mmHg, nd 3 7 mmHg at mean P_{O_2} alue of 546 mmHg, (*b*) for P_{CO_2} with the lectrode 0.3 mmHg at mean P_{CO_2} value of 31 6 mmHg, and ( ) for pH 0 005 at mean pH lue of 7 40.

RESULTS

Various experimental models were used. The design of the experiments and the results are reported in detail in Table I and Figs. 1–3

Oxygen tension

Initially when the mothers were breathing air the mean maternal P_{aO_2} (in arterial blood) was 92 mmHg (range 85–99), and the mean P_{O_2} in amniotic fluid was 13.5 mmHg (range 11 0–14 6). In the following descriptions these values are designated initial

The effect of *hyperoxia* was examined in different ways In 4 cases (nos. 1 2, 7 and 8), the mothers were exposed to a period of hyperoxia (breathing 100 O for 15 min) after the initial measurements. The P_{aO_2} increased to an average of 531 mmHg (range 484–604 measured only in cases 1 7 and 8) and the amniotic fluid P_{O_2} increased in all 4 cases by an average of 9.2 mmHg (range 3.3–20.0). In 2 of these cases the period of hyperoxia was extended to about 1 hour (nos. 7 and 8). The maternal P_{aO_2} remained at an increased and fairly constant level (see note to Table I) The amniotic fluid P_O in the 2 cases showed a further increase from the 15th to the 30th minute of 6.5 and 2.6 mmHg, respectively At 30 min a *steady state seemed to be established as no further* change in amniotic fluid P_{O_2} was noted.

In 2 cases (nos. 5 and 6) the period of hyperoxia (15 min) was preceded by first, a period of hypoxia (10–20 min) and then a recovery period of air breathing (20–25 min). Hyperoxia in these 2 cases was accompanied by an increase of amniotic fluid P_{O_2} above the *recovery value* of 5.5 and 2.6 mmHg, respectively

When hyperoxia was followed by a recovery period of air breathing, the amniotic fluid P_{O_2} returned to the initial level within 30–60 min in 4 cases (nos. 5 6, 7 and 8) but in 1 case (no 2) the P_{O_2} was sti higher than the initial value after 72 min of recovery

The effect of *hypoxia* was examined during first period of moderate hypoxia (breathing 15 O_2) of 10 min which reduced maternal P_{aO_2} to about 60 mmHg. During an ensuing second period of pronounced maternal hypoxia (breathing 10 O_2) of 10 min, a further decrease of maternal P_{aO_2} to about 40 mmHg was noted (measured in nos. 1 and 6). When hypoxia was thus induced in two stages without preceding hyperoxia (nos. 3 4 and 6), the amniotic fluid P_{O_2} decreased on an average by 4. mmHg (range 3 1–5.3) below the initial level. The decrease was more pronounced with 10 O than with 15% O_2. One mother (no 5) was exposed to only one period of hypoxia (10 O) lasting 10 min. In this case the amniotic fluid P_{O_2} decreased by 3 mmHg below the initial level. In another mother (no 1), the two periods of hypoxia were preceded by hyperoxia (15 min) and a recovery period of air breathing (10 min). In this case the amniotic fluid P_{O_2} which had increased during hyperoxia, decreased during hypoxia but not below the initial level.

When hypoxia was followed by a recovery period of air breathing (20-25 min) the amniotic fluid P_{O_2} regained the initial level (nos. 3 5 and 6).

about 30–60 min. During pronounced hypoxia, a significant decrease in amniotic fluid P_{O_2} was noted, and after hypoxia the return to the initial level took about 20–25 min.

There is ample evidence to indicate that inhalation of 100% O_2 by the mother will usually increase P_{O_2} in foetal blood and tissue (1 4, 10 11 13 18 19), even though this may not always be the case (6, 12, 25).

The present experiments do not allow any certain conclusions as to the mechanism whereby amniotic fluid P_{O_2} is influenced by changes in the respiratory oxygen fraction of the mother Obviously one mechanism may be that a change in maternal P_{aO_2} causes changes in the same direction in the foetal blood and tissue P_{O_2}, and that the amniotic fluid P_{O_2} mainly reflects this change. However other explanations such as diffusion of O_2 from maternal tissues cannot be excluded. Although the present results are quite compatible with the hypothesis of Sjostedt et al.—that amniotic fluid P_{O_2} mainly reflects the P_{O_2} of foetal peripheral tissues—they do not prove such a mechanism.

The time course of the change in amniotic fluid P_{O_2} was slow and in terms of the above-mentioned hypothesis, this would probably depend on a slow equilibration between foetus and amniotic fluid. A fluid sample would thus not reflect the immediate state of foetal oxygenation.

The highest amniotic fluid P_{O_2} value noted in the present experiments was 31 mmHg and this value was not exceeded even during prolonged hyperoxia. This observation may indicate the existence of a biological limit, which would be compatible with the hypothesis that amniotic fluid P_{O_2} is mainly influenced by the P_{O_2} in foetal blood and tissue. As pointed out by Assali et al. (2) foetal blood P_{O_2} would only increase to certain level during maternal hyperoxia unless extreme procedures such as hyperbaric oxygen administration to the mother were adopted.

In 2 cases a significant decrease in amniotic fluid P_{CO_2} was observed during hyperoxia. However interpretation of this finding is not clear. In earlier investigations, maternal hyperoxia has been reported to cause decrease in P_{CO_2} of foetal scalp blood (11) but an increase has also been reported (5, 19).

During hypoxia an increasing respiratory alkalosis was noted in maternal arterial blood. This was obviously due to hyperventilation. However no systematic change of P_{CO_2} or pH in amniotic fluid was noted during maternal hypoxia.

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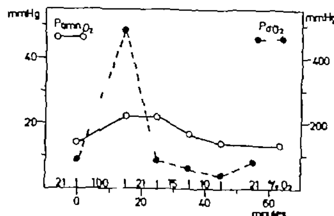


Fig 1 Case 1 Oxygen tension in maternal arterial blood (Pa_{O_2}) and amniotic fluid ($Pamn_{O_2}$) during normoxia, induced maternal hyperoxia and hypoxia. The fraction of inspired O_2 is given on the abscissa. Open circles and full lines indicate $Pamn_{O_2}$ (left ordinate). Filled circles and broken lines indicate Pa_{O_2} (right ordinate)

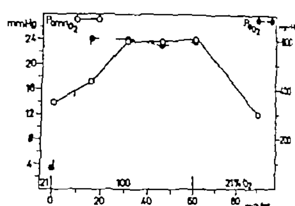


Fig 3 Case 7 Oxygen tension in maternal arterial blood (Pa_{O_2}) and amniotic fluid ($Pamn_{O_2}$) during normoxia and induced maternal hyperoxia. The fraction of inspired O_2 is given on the abscissa. Open circles and full lines indicate $Pamn_{O_2}$ (left ordinate). Filled circles and broken lines indicate Pa_{O_2} (right ordinate)

Maternal acid–base status

Initially the following mean values were found in maternal arterial blood P_{aCO_2} 33 mmHg, pH 7 42, standard bicarbonate 22.1 mmol/l, and base excess −2.9 mmol/l. These values give the picture of a metabolically compensated respiratory alkalosis of moderate degree.

During *hyperoxia* a slight decrease of P_{aCO_2} and a slight increase of pH was seen (4 out of 5 cases) but there was no change of standard bicarbonate or base excess.

During *hypoxia* P_{aCO_2} decreased and pH increased in all cases examined, but no change in standard bicarbonate or base excess was seen.

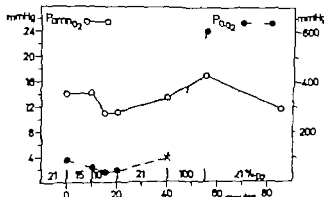


Fig 2 Case 6 Oxygen tension in maternal arterial blood (Pa_{O_2}) and amniotic fluid ($Pamn_{O_2}$) during normoxia, induced maternal hypoxia and hyperoxia. The fraction of inspired O_2 is given on the abscissa. Open circles and full lines indicate $Pamn_{O_2}$ (left ordinat). Filled circles and broken lines indicate Pa_{O_2} (right ordinate).

Amniotic fluid P_{CO_2} and pH

Initially the mean values were for P_{CO_2} 46.4 mmHg and for pH 7 15

During *hyperoxia* a decrease of pH was noted in 1 case after 30 min without any significant change of P_{CO_2} (no. 7) This was the only case with a slight increase of maternal P_{aCO_2} during hyperoxia. In the other cases no significant pH changes were noted. In 1 case (no 5) a decrease of P_{CO_2} was observed during hyperoxia preceded by hypoxia and a recovery period. In another case (no 8) a decrease of P_{CO_2} was noted during extended hyperoxia and the decrease remained during 60 min of recovery with air breathing.

During *hypoxia* no changes of P_{CO_2} or of pH were noted.

DISCUSSION

The results of the present investigation demonstrate that in the second trimester of human pregnancy the oxygen tension of amniotic fluid can be significantly influenced by such changes in the inspiratory oxygen fraction of the mother as are sufficient to cause a considerable change in maternal arterial P_{aO_2}. Maternal hyperoxia always induced a rise in the amniotic fluid P_{O_2}, while maternal hypoxia of pronounced degree induced a fall in the P_{O_2}.

The time course of the amniotic fluid change was slow During maternal hyperoxia, amniotic fluid P_{O_2} reached its maximum level after 15 min and before 30 min of hyperoxia. Usually after hyperoxia, the return to the initial level was complete within

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MORPHOLOGY OF THE FETAL ADRENAL CORTEX, AND MATERNAL URINARY OESTRIOL EXCRETION IN PREGNANCY

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Abstract. A morphological examination of the fetal zone of the adrenal cortex has been carried out in 562 controls and 58 pathological cases, and maternal urinary oestriol excretion has been determined during the pregnancy, in order to elucidate the role of the fetal adrenals. The pathological cases consisted of infants of mothers with Rh-immunization, diabetes mellitus and toxaemia, together with a small group without any common features other than low oestriol excretion.

The adrenal weight was found to be correlated to the normal urinary oestriol excretion, and to a lesser degree to reduced excretion.

Both in Rh-immunization and diabetes mellitus the degree of necrosis in the fetal zone was increased, while the amount of haemorrhage did not differ from the controls. Moreover, in Rh-immunization an accumulation of lipid in the fetal zone was found. The morphological changes were related to the maternal oestriol excretion, and to the concentration of haemoglobin in infants of mothers with Rh-immunization, but not to the severity of the maternal diseases.

In toxaemia only cases with reduced maternal oestriol excretion differed from the controls, and both increased haemorrhage and necrosis were found, without any relation to the degree of toxaemia. In three toxaemic cases the width of the foetal zone was reduced. This was not found in any of the other groups. The rest showed massive necrosis of the foetal zone in three cases, lipid accumulation in one, and hypoplastic adrenals in one.

The importance of the morphological findings in the fetal adrenals of infants of mothers with Rh-immunization, diabetes mellitus and toxaemia is discussed.

Since Elliot & Armour (16) described the fetal zone of the adrenal cortex, many investigations have been published about the morphology and the involution of this zone (23, 25, 28). In 1969 we described the involution as a haemorrhagic necrosis without inflammatory reaction, starting before birth, but chiefly setting in within the first week of extra-uterine life (2).

In 1956 Benirschke demonstrated adrenal atrophy in anencephalic monsters, and in 1961 Frandsen & Stakemann found a low level of maternal urinary oestriol excretion in anencephaly. Over the last few years the maternal urinary oestriol excretion has been employed as a test of function of the feto-placental unit (8, 14, 17, 18, 22, 43). As we had the opportunity of obtaining specimens from infants who had died in the neonatal period and whose mothers had determinations of the urinary oestriol excretion carried out in pregnancy, we considered it of value to examine the morphology and the involution of the fetal adrenal cortex partly in a near normal group, and partly in a group of pathological cases, where the maternal urinary oestriol excretion had been determined.

MATERIAL AND METHODS

The controls consisted of 562 adrenals from 562 fetuses and infants ranging in age from 11 weeks of gestation to 2 years after delivery. The material was selected from a continuous autopsy series of 782 specimens, from which cases of low maternal urinary oestriol excretion, Rh-immunization with Rh-positive fetuses, diabetes mellitus, toxaemia, and cases of hydrops fetalis, chromosomal abnormalities, kernicterus, anencephaly and generalized infections together with cases of maceration were excluded. In this way the selected material was from virtually normal pregnancies, and was composed of 126 stillborn and 436 live born infants. The stillborn infants were classified according to the age of gestation estimated by means of the foot length, while the live born infants were classified according to their age at subsequent death. In 25 cases the maternal urinary oestriol excretion was determined.

The pathological series consisted of 58 cases in which the maternal urinary oestriol excretion was determined.

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found signs of inflammation during the involution of the fetal zone. In the live born infants the de gree of involution was not related to the gestational age t birth. Centrally located vacuoles were seen both in the stillborn and in the live born infants, but they disappeared during the first month after delivery The vacuoles were stained with the lipid stains.

The width of the fetal zone (Fig. 3) was 88 ± 10% ($p < 0.05$) of the total cortex early in fetal life, and in the first day after delivery 82 ± 16% After the second day the zone constituted a decreasing part of the total cortex, and in infants 1 2 years of age the zone made up less than 19%

The relative adrenal weight was found to be largest in the middle of fetal life (0.38 ± 0.33% $p < 0.05$), and was only slightly reduced until the fourth day after delivery After this time the adrenals made up a decreasing part of the body weight, until in infants 1–2 years of age the adrenal weight was less than 0.13% of the body weight.

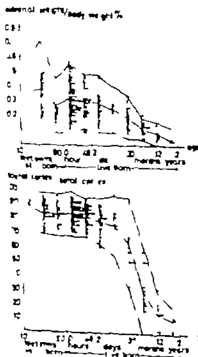


Fig. 3 The relation of the age to the relative adrenal weight in 446 infants and to the width of the foetal zone in 54 infants. The mean values and the range within 1.96 S.D. (p 0.05) is indicated

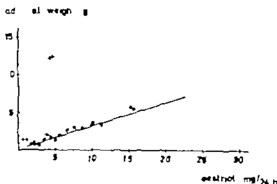


Fig. 4 The relation of the adrenal eight to the oestriol excretion. The normal oestriol values are indicated as (O). The slope of the regression line is 0.31 and the correlation coefficient 0.67 The low oestriol values are indicated as (+), and the correlation coefficient is 0.47

In 25 cases the maternal urinary oestriol ex cretion was determined. On comparing the mor phology of the fetal adrenals in this group with the other controls we found no difference. We therefore considered it justifiable to use all the controls as reference material, though the oestriol excretion had been determined in so few Fig. 4 shows the relation f the adrenal weight to the maternal oestriol excretion (correlation coefficient 0.67)

2. *The pathological series*

We examined the cases with reduced maternal oestriol excretion as a whole, and then all the cases of maternal Rh-immunization, diabetes mellitus, toxaemia and the remaining 5 cases were studied as separate groups.

Low maternal oestriol excretion (76 cases). The results of the morphological investigation appear in Fig. 5 Comparing this with controls we found no difference in the occurrence and degree of haemorrhage, but, on the other hand, massive necrosis was found in about half of the stillborn and live born infants dying within the first 2 days after the delivery and in all the cases in the older age groups. The correlation coefficient between the adrenal weight and the reduced values of oestriol was 0.47 (Fig. 4).

Rh-immunization (22 cases). This group did not differ from the controls in the degree of haemor rhage in the fetal zone, while necrosis was more pronounced, and most marked in those cases with low maternal oestriol excretion. Furthermore, in

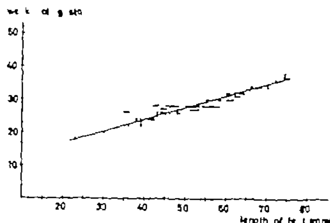


Fig 1 The relation of the foot length to the weeks of gestation of 189 stillborn infants. The slope of the regression line is 0.29 and the correlation coefficient 0.74

18 cases (31%) were stillborn. The values of oestriol excretion were below normal in 26 cases. In 22 cases the mothers had clinically known Rh-immunization with Rh-positive fetuses. The oestriol excretion was reduced in eight of these cases and three mothers delivered stillborn infants. In 20 cases the mothers had diabetes *mellitus requiring insulin treatment*. The disease was classified according to White et al. (42). Seven diabetic cases had reduced oestriol excretion, and four mothers delivered stillborn infants. In 11 cases the mothers had toxaemia defined as blood pressure above 140/90, proteinuria, and oedema. The disease was classified as preeclampsia or eclampsia. In 6 of these cases the oestriol excretion was reduced, and 7 mothers delivered stillborn infants. The remaining 5 cases were a mixed group without any common feature other than a reduced maternal oestriol excretion. Four of these mothers had stillborn infants. The infants in the pathological series varied in age from fetuses with a foot length of 31 mm to infants who died 10 days after delivery

The maternal urinary oestriol excretion was determined in mg/24 hrs and only cases with at least two determinations were included in this study, the last one being *performed within 5 days of delivery*. The oestriol excretion was classified according to the diagram of Frandsen (17) in a group with values within the normal range and a group with values below. (The urinary oestriol was measured at Statens Seruminstitut, Hormonal Department, Copenhagen.) The age of gestation was estimated by means of Fig. 1

The adrenals were removed 6 to 24 hrs post mortem and fixed in 10% buffered formalin. Haematoxylin-eosin and van Gieson stained paraffin sections have been made in all cases, and also supplied with Sudan III, Oil red O and Scarlach Red stained frozen sections. Each specimen was examined for the occurrence of inflammation, haemorrhage, necrosis, and lipid content in the fetal zone of the adrenal cortex. The haemorrhage and the necrosis were defined and graded as described earlier (2), and the lipid content was graded in four degrees also.

The fetal cortex was measured in arbitrary units in relation to the total cortex, and the combined infant adrenal weight was indicated as absolute and relative weight in relation to the body weight of the infant.

RESULTS

1 *The controls*

The results of the morphological examination appear in Fig. 2. Among the stillborn and the live born infants dying within the first day we found only a slight degree of haemorrhage located in the fetal zone. However in 14 cases (2.5%) massive haemorrhage of both the fetal and the permanent zone was seen. In cases of death after the second day of life the haemorrhage became accentuated, and was most marked in infants who had survived for a month. Among the stillborn and live born infants dying within 4 days of delivery massive necrosis was found in 4–5% while a moderate degree of necrosis was found in about one-third. When death followed the fourth day we saw massive necrosis more frequently in the fetal zone, and at the end of the first year the zone was totally necrotic. We never

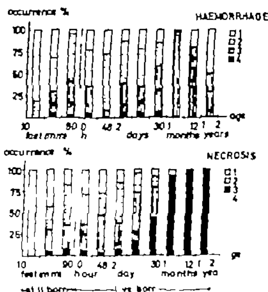


Fig 2 The distribution according to age and degree of haemorrhage and necrosis in the fetal zone of the adrenal cortex of 56_ stillborn and live born infants ranging in age from 10 mm foot length to 2 years after the delivery. The degree of haemorrhage is graded as follows (1) No haemorrhage (2) haemorrhage in the centre of the fetal zone (3) haemorrhage in the whole fetal zone and (4) massive haemorrhage in both the fetal and the permanent zone. Necrosis is graded as (1) no necrosis, (2) necrosis in the central part, (3) central necrosis combined with focal peripheral necrotic parts, and (4) massive confluent necrotic parts in the whole fetal zone.

are the limiting factor in the feto-placental unit in the production of oestrogen (4 5 18, 22) Fur thermore, the urinary oestriol excretion in pregnant women was found to be related to the weight of the placenta and the birth weight (22, 29 37). Frandsen (17) found a strong resemblance between the curve of the mean oestriol excretion and the fetal adrenal weight, which we were able to erify In this investigation the mor phological features of the fetal adrenals in cases of low maternal oestriol excretion were found to differ remarkably from the controls, while the relative adrenal weight was within the normal range. Furthermore, the correlation coefficient was 0 67 in the controls but only 0.47 in cases of low oestriol alues. This indicates that the fetal adrenals are only one of the factors regulati g the amount of the produced and excreted oestrogens, and that a low oestriol excre tion does not ha e to be reflected in an adrenal hypoplasia as in anencephaly

Taylor et al. (41), in 6 patients with Rh-immunization, found low levels of urinary oestriol excretion in cases of stillborn infants, while the remaining majority of the rinary oestriol levels, xcept in one case, were considerably greate than those found in pregnant women without complications. Liley (30) has mentioned an increase in the oestriol concentration in the amniotic fluid in cases of Rh-immunization, and Mandelbaum et al. (34) found, in 43 patients, that severe erythroblastosis was sociated with depression of maternal rinary oestriol excretion, while low oestriol levels rose when fetal survival occurred after intra-uterine transfusions. In accordance with this we found, in infants of mothers with Rh-immunization, that the most pronounced morphological changes in the fetal adrenal zone occurred in cases of low maternal oestriol ex cretion

Lipid accumulation in adrenals in cases of erythroblastosis has been demonstrated in several investigators (1 6, 10, 12). Some ha e shown relation between the lipid accumulation and the degree of anaemia in the child (1 6), which was lso found in this study while Claireaux (10) found lipid accumulation only in cases of hydrops f talis. Pearson et al. (38) has published a case of nonimmunological a-thalassaemia with extens lipid accumulation in the adrenals. This may indicate that the immunological circumstances do not determine the adrenal changes in erythroblastosis, but rather the intra-uterine haemolysis itself In accordance with this we did not find any relation between the morphological changes in the fetal adrenals and the degree of maternal immunization. Bartman & Driscoll (1) assumed that the fetal adrenals were stimulated to hyper function in the preterminal stage of erythroblastosis. By this suggestion the clinical and the morphological findings could be explained. Thus, the increased adrenal activity in the early stage of erythroblastosis may be reflected in an increase of maternal oestriol excretion. In severe erythroblastosis the foetal adrenals may be exhausted, manifested clinically in a depression of the oestriol excretion and morphologically in a massive necrosis and lipid accumulation in the fetal zone of the adrenal cortex.

Some investigations of oestriol excretion in pregnant diabetics have shown no tendency to low levels of oestriol compared with normals (13, 19 21 29). Yet Lyngbye (32) found a significant reduction of urinary oestriol, 17β-oestradiol and pregnanediol. Smith et al. (40) found a different response to the respiratory distress syndrome in infants of diabetic mothers compared with normal infants, as they showed a significant increase in urinary 17-hydroxycorticosteroids, whereas infants of non-diabetic mothers did not. Cathro & For syth (9) in 8 newborn infants of diabetic mothers showed tendency to increased excretion of corticoids and certain 17-ketosteroids suggesting an increase in adrenal activity in these infants. On the other hand, Migeon et al. (35) and Aarskog (45) found that the levels of 17-hydroxycorticosteroids and total cortisol in cord plasma did not differ significantly from those of normal infants. Further more, Cleary & Pion (11) revealed a marked reduction in 16 a-hydroxydehydroisoandrosterone excretion in a newborn baby of a diabetic mother which may reflect poor hydroxylation in the fetal li er

Morphological findings in the fetal adrenals of infants of diabetic mothers have differed as well. Some found enlarged adrenals (23 26) and Naeye (36) demonstrated that this was mainly due to an increased fetal zone. Others could not verify this (7 15). W found slightly increased necrosis in the fetal zone which was most marked in cases with low maternal urinary oestriol ex cretion, but without any relation to the degree

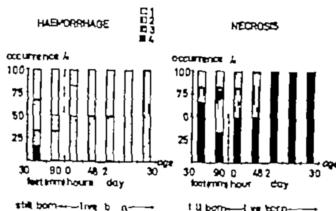


Fig. 5. Distribution according to age and degree of haemorrhage and necrosis in the fetal adrenal cortex of cases with low maternal urinary oestriol excretion. Haemorrhage and necrosis are graded as in Fig. –.

the whole group we found an increased content of lipid, which was most obvious in the cases with low oestriol excretion too. Six of the 8 cases in this group had a massive accumulation of lipid, while we found only one case among the group with normal oestriol excretion with as much lipid as this. No signs of inflammation were found. The morphology of the fetal adrenals was not related to the degree of the maternal Rh-immunization as measured by the indirect Coombs test. On the other hand, both the degree of necrosis and the lipid content were related to the degree of erythroblastosis as measured by the haemoglobin level in the infants. The relative adrenal weight and the width of the fetal zone were within the normal range.

Diabetes mellitus (20 cases). This group did not differ from the controls in the occurrence and degree of haemorrhage in the fetal zone. However, we found a tendency to a more pronounced degree of haemorrhage after delivery among the cases with normal maternal oestriol excretion. On the other hand, the degree of necrosis differed slightly and was most marked in cases of low oestriol excretion. The content of lipid was normal, and no signs of inflammation were found. The severity of the diabetes did not influence the morphological features in the fetal adrenals. The relative adrenal weight and the width of the fetal zone were within the normal range except in one case with hyperplastic adrenals. The maternal oestriol excretion in this case was normal.

Toxaemia (11 cases). In the cases with normal maternal oestriol excretion the morphology of the fetal adrenals did not differ from the controls, but in cases with reduced oestriol excretion we found more pronounced haemorrhage and necrosis in the fetal zone. The content of lipid was normal, and no signs of inflammation were found. The adrenal changes were not related to the severity of the maternal toxaemia. The relative adrenal weight was within the normal range while the width of the fetal zone was reduced in 3 cases with a low maternal urinary oestriol excretion.

The remaining cases (5 cases). In this group there was a maternal history of pulmonary embolism, acromegaly and abruptio placentae in 3 cases while the others had no previous abnormality. Autopsy of the infants showed cardiac malformations in 2 cases, while the rest revealed nothing particular. By comparing them with the controls we found 3 cases with massive necrosis of the fetal zone, one case with lipid accumulation, and one with adrenal hypoplasia.

DISCUSSION

The physiological involution of the fetal zone of the adrenal cortex was found to proceed as described earlier (2). Yet we found massive adrenal haemorrhage more frequently in this investigation, and furthermore centrally located lipid both before and during the involution was a normal feature of the fetal cortical cells.

The synthesis of oestriol during pregnancy is assumed to start in the placenta from pregnenolone and progesterone, which are transferred to the fetus to undergo a hydroxylation and sulphurylation, and transformed to dehydroepiandrosterone and its sulphate in the fetal adrenals. In the fetal liver 16α hydroxylation takes place and the steroids are then transported back to the placenta, where the sulphate is removed, and the steroids are aromatized to yield oestriol and the other oestrogens (14, 43, 44).

It is well-known that the urinary excretion and concentration of oestrogens in the cord and the maternal plasma are at a low level in anencephaly (20, 31, 37). Benirschke (3) demonstrated fetal atrophy in this condition due to a premature involution of the fetal zone after the twentieth week of gestation, and Maeyama et al. (33) found no increase in oestriol after ACTH administration to mothers of anencephalic monsters.

Several authors assume that the fetal adrenals

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of the maternal diabetes. The relative weight of the adrenals and the width of the *fetal zone* were within the normal range except in one case.

The varying results of morphological investigations may be explained by the fact that most of the infants in our series and those of Cardell (7) and Driscoll et al. (15) were delivered before term. Studies of steroid metabolism in these infants may be explained by assuming an increased adrenal activity secondary to a possible hepatic enzymatic deficiency and an induced hyperglycaemia as suggested by Pedersen (39) in cases of normal maternal urinary oestriol excretion. On the other hand, cases of low maternal oestriol excretion reflect a more severe affection of the fetal adrenals, possibly because of increased demands on steroid production, though the morphology of the adrenals were not related to the severity of the maternal diabetes.

Lenters (27) and Liggins & Evans (29) found reduced urinary oestriol excretion in mothers with toxaemia of pregnancy and Lenters demonstrated a relation between the oestriol values and histological changes in the placenta. On the other hand Bręborowicz et al. (5) could not explain the low values of oestrogen by the size or the histological findings in placentas in this condition. Moreover Dickey & Robertson (13) did not find a significant correlation between newborn oestrogen excretion and the degree of toxaemia. Laumas et al. (24) examined in vitro the biosynthesis of oestrogens in microsomal fractions obtained from placentas from seven toxaemic cases, and found that the conversion of 19-hydroxyandrostenedione into oestrogen was reduced compared with the normal placenta. As 19-hydroxylation is assumed to be the limiting step in aromatisation the reduced excretion in toxaemia can be regarded as being caused by a placental defect. Cleary & Pion (11) examined C_{19}-steroid excretion in 5 infants of toxaemic mothers with low oestriol excretion in pregnancy and found a reduction of 16α hydroxyisoandrosterone indicating an enzymatic deficiency of the fetal liver. Examination of the fetal adrenals in our investigation revealed increased haemorrhage and necrosis in the fetal zone in cases of low maternal oestriol excretion associated with toxaemia. The morphological changes were not related to the severity of the maternal toxaemia, but in cases of normal oestriol excretion the morphology did not differ from the controls. However this group only consisted of 11 cases. Yet in 3 cases the width of the fetal zone was reduced, which is remarkable as we did not find this in the other groups. The morphology of the fetal adrenals in toxaemia may be explained as secondary to possible enzymatic deficiencies of both the fetal liver and the placenta.

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UTERO-VAGINAL DIFFERENTIAL PRESSURE DURING THE FIRST STAGE OF LABOUR

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Abstract. Intra-abdominal (vesical), intra-uterine and intra-vaginal pressures were measured in 12 healthy women early in the first stage of labour. Ten subjects were primigravidae and two gravidae-2.

Seven subjects exhibited no significant changes of intra-abdominal or intra-vaginal pressures during uterine contractions.

Five subjects (4 primigravidae) exhibited no significant change of intra-abdominal pressure but a large and significant increase of intra-vaginal pressure concomitant with uterine contractions. In spite of having higher intra-uterine peak pressures during contractions, these subjects exhibited significantly smaller expulsive uterine pressures than the former group because of high vaginal counter-pressures. It is believed that increased tension of pelvic floor muscles contributes to the elevated vaginal pressure during contraction.

Our findings may offer a better understanding of why pain relief in selected patients will aid in accelerating labour and make the birth process less hazardous for the fetus.

Nearly a century ago Schatz (1872) made the first recordings of intra-uterine pressure and suggested that the bladder be used for evaluation of intra-abdominal pressure. This idea was later taken up by Alvarez & Caldeyro (1950). Recently evidence has been presented that intra-vesical pressure closely reflects intra-abdominal pressure (Söderberg & Westin, 1970). Evidence has also been presented that rapid and complete transmission of pressure occurs from the peritoneal to the uterine cavity and nearly complete transmission of pressure from the peritoneal cavity to the part of vagina situated cranially to the pelvic floor (Söderberg & Westin, 1969).

The expulsive uterine pressure is the difference between the intra-uterine and the intra-vaginal pressures. In uterine patients with a low pain threshold it seems possible that, because of fear, contraction of pelvic floor muscles might diminish the expulsive uterine pressure.

The present study is a preliminary report on this subject.

MATERIAL AND METHOD

Twelve healthy full-term women early in the first stage of labour were randomly selected for study. 10 subjects were primigravidae and 2 were gravidae-2. The presenting part of the fetal head was the occiput. The station was above or at the spines. Cervical dilatation varied from between 2 and 6 cm. The subject was asked to void. Thereafter catheters for pressure recording were introduced into the bladder, the amniotic cavity and into the top of the vagina immediately below the presenting part as described in detail earlier (Söderberg & Westin, 1969). Recordings were performed with the patient in the supine position. Analyses of the recorded pressures in the three compartments were performed when the intra-uterine pressure was lowest between contractions and again at the peak of uterine contractions.

RESULTS

Fig. 1 illustrates the effect of coughing, bearing down and of contraction of the pelvic floor muscles on the utero-vaginal pressure gradient in the same patient. The rapid pressure changes induced by coughing did not appreciably alter the utero-vaginal pressure difference but there was a change when the patient was bearing down. Pelvic floor contraction caused a pressure gradient in the opposite direction, the pressure increase in the top of the vagina being about twice as high as that in the uterus.

The mean pressures between, and at, the peak of uterine contraction for the three sites in the 12 patients are illustrated in Fig. 2. The intra

Gynaecologists

Menorrhagia may be caused by an increase in local fibrinolytic activity Cyklokapron reduces menorrhagic haemorrhages by an average of 50%

Women with average menstrual blood losses of over 80 ml have higher concentrations of plasminogen activators in the endometrium than those with lower blood losses. The resultant increase in local fibrinolytic activity is inhibited by Cyklokapron The recommended dosage of Cyklokapron in menorrhagia is 1 g 3–6 times daily for 3–6 days. With a dosage of 3 g daily Nilsson and Rybo noted reductions in bleeding of 38 % compared with control cycles. With twice this dosage bleeding was reduced by 51 %. None of the 36 patients participating in the trial were obliged to discontinue treatment as a result of side-effects

Reference. NILSSON L. RYBO G Treatment of menorrhagia with an antifibrinolytic agent, tranexamic acid (AMCA) A double blind investigation Acta Obstet. Gynecol Scand. 46 (1967) p 572

the fibrinolytic system

ACTIVATORS
tissue activators
lab blood activators
streptokinase
urokinase
trypsin

plasminogen
plasmin

INHIBITORS
inhibitors of plasminogen activators
(= urokinase inhibitors)
EACA AMCA
α2 – macroglobulin
α1 – antiplasmin

fibrinogen
fibrin
factor V
AHF
other proteins

Split products
HMWS
D E

compartments as described in Fig. 1. After suggesting to the patient that she relax, all pressures are lowered and the pressure peaks were abolished. The previous condition could immediately be induced again by asking the patient to react to her contractions as she had before relaxation.

DISCUSSION

The preliminary results of this paper seem to indicate that vaginal counterpressure, probably in part brought about by increased tension of pelvic floor muscles, may be common in patients with fear and tension during labour. Although this series is too small for definite conclusions to be drawn, it seems reasonable to assume that reduction of the expulsive uterine pressure, partly caused by pelvic floor contraction, might make labour more difficult and prolonged. These ideas fit well with the findings of Engstrom et al., 1964 according to whom patients with adverse psychosocial factors more frequently have prolonged labour. The high intra-uterine pressure will also decrease the perfusion pressure in the arteries supplying the placenta (Woodbury et al., 1938) and might thus endanger the fetus.

Psychological preparation for childbearing particularly shortens the latent and acceleration phases (Friedman, 1955; Da is & Morrone, 1962)

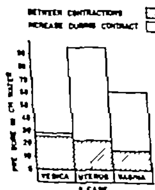


Fig. 4. M an. shows between and during uterine contractions: intra-abdominal, intra-uterine and intra-vaginal pressures in 3 subjects exhibiting no significant increase ... abdominal but large and significant increase of ... vaginal pressure during contraction. In spite of very high intra-uterine pressure at the peak of uterine contraction the expulsive uterine pressure (mean 35 cm of water) ... significantly lower than in Fig. 3.

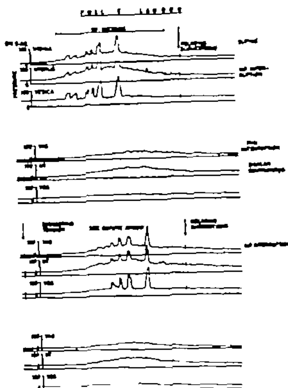


Fig. 5. The effect of verbal suggestions on intra-abdominal, intra-uterine and intra-vaginal pressure pattern in ... multipara during the first stage of labour. The rapid and large pressure changes which are repeatedly seen in connection with contraction are of abdominal origin and interpreted as Valsalva manoeuvres. The high vaginal counterpressure may depend, in part, on contraction of pelvic floor muscles (see Fig. 1). Relaxing suggestions abolished the abdominal pressure peaks and were accompanied by lowering of pressures in all three compartments both between and during uterine contraction.

while light medication (Friedman, 1955) shortens the active phase during the first stage of labour.

If our preliminary findings hold true they will offer better understanding of why pain relief in selected patients will aid in accelerating labour and making the birth process less hazardous for the fetus. In the tense patient there is reason to believe that pain relief will decrease intra-uterine pressure, thereby increasing the placental perfusion pressure, and, simultaneously the expulsive intra-uterine pressure will increase.

ACKNOWLEDGEMENT

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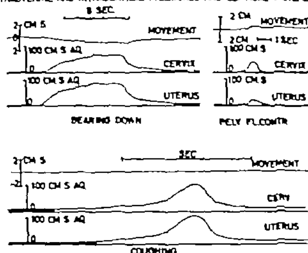


Fig. 1. Intra-uterine and intra-vaginal pressures and cervical movement in early first stage of labour in a nullipara. The intra-vaginal pressure was measured at th. external os of the cervix. Cervical movement was related t a fixed point on the bony pel is. The one end of a light plastic rod was attached to the cervix with a clip. The other end was attached to a Wheatston. bridge whereby a linear movement of the cervi could be presented in a linear fashion on the recording paper. During coughing there was practically no change in utero-vaginal pressure relationship. Bearing down, probably by relaxing the pelvic floor muscles, caused a small uterine expulsion pressure (utero-vaginal pressure difference). Pelvic floor contraction caused a pressure increase in the top of the vagina twice as pronounced as that in the uterus.

abdominal (vesical) pressure increase is insignificant while the corresponding increase in intra-vaginal pressure is of some significance ($0.1 > p > 0.05$).

A further analysis of the cases made it clear

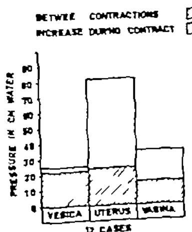


Fig. 2. Mean values for all 1 subjects between and during uterine contractions f intra-abdominal (vesical), intra-uterine nd intra- aginal pressures.

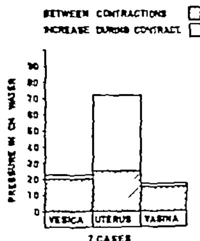


Fig. 3. Mean values between and during uterine contractions of intra-abdominal, intra-uterine and intra-vaginal pressures in 7 subjects exhibiting no significant increase of intra-abdominal or intra- aginal pressures during uterine contractions. The mean uterine xpulsi pressure (utero-vaginal pressure difference) is 55 cm of ater.

that two different types of pattern occurred (Figs. 3 and 4). In 7 patients only small and insignificant (range 0–10 cm water) changes of pressures occurred in the abdominal cavity and the vagina during uterine contractions (Fig. 3). In 5 patients a similar small pressure change occurred in the abdominal cavity while a large and significant ($0.0? > p > 0.01$) increase in intra-vaginal pressure took place concomitant with uterine contractions (Fig. 4). The expulsive uterine pressure was significantly smaller than in the former group (Fig. 3) because of the large vaginal counterpressure. Four of these patients were deprimigravidae and two of them had to be delivered by vacuum extraction. The general impression was that all 5 of these patients seemed to suffer more from the labour pains than the others.

That verbal suggestions of relaxation and of breathing freely without any previous training can modify the pattern of the pressure curves in all compartments, is illustrated in Fig. 5. The sets of records at the top of the figure show a series of sudden, large pressure peaks emanating from the abdominal cavity and transmitted to the uterus and the agina. These pressure peaks are interpreted as Valsalva manœuvres as the patient was not seen to be bearing down. The high intra-uterine pressure is derived to some extent from the abdominal cavity but largely by transmittance of pressure between the vaginal and uterine

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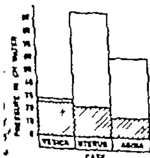


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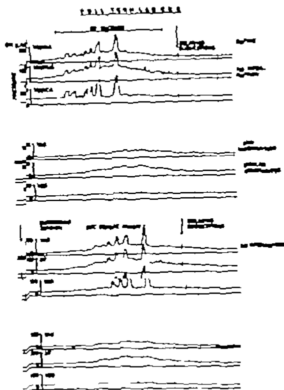


Fig. 3. The effect of verbal suggestions on intra-abdominal, intra-uterine and intra-vaginal pressure pattern in a primipara during the first stage of labour. The rapid and large pressure changes which are repeatedly seen in connection with contraction are of abdominal origin and interpreted as Valsalva manoeuvres. The high vaginal counterpressure may depend, in part, on contraction of pelvic floor muscles (see Fig. 1). Relaxing suggestions abolished the abdominal pressure peaks and were accompanied by lowering of pressures in all three compartments both between and during uterine contractions.

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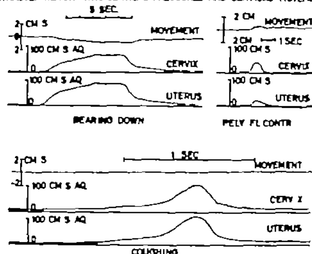


Fig 1 Intra-uterine and intra-vaginal pressures and cervical movement in early first stage of labour in a nullipara. The intra-vaginal pressure was measured at the external os of the cervix. Cervical movement was related to a fixed point on the bony pelvis. The one end of a light plastic rod was attached to the cervix with a clip. The other end was attached to a Wheatstone bridge whereby a linear movement of the cervi could be pre sented in linear fashion on the recording paper During coughing there was practically no change in utero-vaginal pressure relationship. Bearing down, probably by relaxing the pelvic floor muscles, caused a small uterine expulsion pressure (utero-vaginal pressure difference). Pelvic floor contraction caused a pressure increase in the top of the vagina twice as pronounced as that in the uterus.

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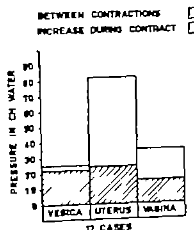


Fig Mean values for all 12 subjects between and during uterine contractions of intra-abdominal (vesical), intra-uterine and intra-vaginal pressures.

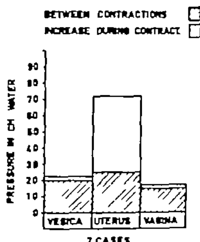


Fig 3 Mean values between and during uterine contractions of intra-abdominal, intra-uterine and intra-vaginal pressures in 7 subjects exhibiting no significant increase of intra-abdominal or intra-vaginal pressures during uterine contractions. The mean uterine expulsion pressure (utero-vaginal pressure difference) is 55 cm of water.

that two different types of pattern occurred (Figs 3 and 4) In 7 patients only small and insignificant (range 0–10 cm water) changes of pressures occurred in the abdominal cavity and the vagina during uterine contractions (Fig. 3). In 5 patients a similar small pressure change occurred in the abdominal cavity while a large and significant ($0\ 02 > p > 0.01$) increase in intra-vaginal pressure took place concomitant with uterine contractions (Fig. 4) The expulsive uterine pressure was significantly smaller than in the former group (Fig. 3) because of the large vaginal counterpressure. Four of these patients were primigravidae and two of them had to be delivered by vacuum extraction The general impression was that all 5 of these patients seemed to suffer more from the labour pains than the others.

That verbal suggestions of relaxation and of breathing freely without any previous training can modify the pattern of the pressure curves in all compartments, is illustrated in Fig. 5 The sets of records at the top of the figure show a series of sudden large pressure peaks emanating from the abdominal cavity and transmitted to the uterus and the vagina. These pressure peaks are interpreted as Valsalva manœuvres as the patient was not seen to be bearing down. The high intra uterine pressure is derived to some extent from the abdominal cavity but largely by transmittance of pressure between the vaginal and uterine

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THE URINARY EXCRETION OF CHORIONIC GONADOTROPHINS AFTER INDUCED ABORTION

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Abstract. The urinary excretion of chorionic gonadotrophins after legal abortion has been followed using an immunological pregnancy test (Pregnosticon®). The test only became negative after several days, and 25% of the cases still had positive tests 11 days after the evacuation of the ovum.

The prognosis for a pregnancy complicated by bleeding during the first trimester is very difficult to evaluate. It is most important to decide if a live foetus is present, but unfortunately our present methods are of only limited value during this period. With modern instruments heart sounds can be heard at 13–14 weeks gestation, but only a positive observation is decisive. Estimation of the maternal excretion of oestrogens seems promising (2, 3) but is not yet widely used.

Most often one has to rely on simple pregnancy test when assessing the viability of the ovum. Such tests depend on the urinary excretion of chorionic gonadotrophins, but a positive test does not mean that living foetus is present, only that some viable placental tissue remains. Clinical experience seems to indicate that not even this is always the case. In the literature a few reports have been published showing that pregnancy tests can remain positive for several days after the uterus has been thoroughly emptied, but few investigations have been carried out.

Møller et al (6) reported 5 cases of abortion where the test (Pregnosticon®) was positive 3 days after the abortion. Schmidt (4) recorded a case where a patient 4 week pregnant had hysterectomy performed, and the pregnancy test still positive 2 weeks later. Allison (1) estimated the excretion of chorionic gonadotrophins, using the quantitative toad test, in women with first trimester abortions. On day 6 post abortem the concentration was 1 600–2 400 I.U./1 000 ml in 4 out of 8 cases, and this concentration was also found in one of 13 cases on day 7.

Thus it seems that the pregnancy test can be positive several days after an intra-uterine pregnancy has been evacuated. This brings up the clinical problem of the meaning of a positive test some days after an evacuation, where only decidual tissue and no placental tissue was removed. Is it indicative of placental tissue outside the uterus, indicating an extra-uterine pregnancy?

For these reasons we found it of interest to investigate how long a positive test could be found after the uterus had been emptied in cases of legal abortion.

MATERIAL AND METHODS

Thirty-two women had legal abortions for psychiatric reasons. All had gestation period of 8–1[illegible] weeks. The uterus was emptied by aspiration, followed by curettage to make sure the uterus was completely empty. In all cases an intra-uterine pregnancy was found.

An immunological test (Pregnosticon®) was used. This test is positive when the concentration of gonadotrophins is above 1 500 I.U./1 000 ml. All tests were carried out on morning specimens of urine, but as some patients mailed the specimens to the hospital, some tests were carried out the day after the urine was voided.

Because of the psychiatric status of the patients it was found rather difficult to persuade them to send urine specimens when they had been discharged from hospital.

RESULTS

From Table I it will be seen that 5 days after the evacuation all 22 cases investigated had posi-

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EXPERIENCE OF THE USE OF DESAMINO-OXYTOCIN BUCCAL TABLETS AS A METHOD OF INDUCING LABOUR, AND OF A SCORING SYSTEM FOR EVALUATION OF READINESS FOR LABOUR

Jarmo Laine

From Department I of Obstetrics and Gynaecology (Head: Keijo Sarva), Tampere Central Hospital, Tampere, Finland

Abstract. Induction of labour was performed on 259 parturients by means of desamino-oxytocin (ODA 914) buccal tablets. The maximal dose was small (350 I.U.). Primary induction was successful in 84% of the cases. The incidence of maternal complications was small.

Neonatal asphyxia was diagnosed in 12 newborn infants (4.6%). Asphyxia was more common in those cases in which toxaemia was the indication for induction. Intrapartum and neonatal mortality were nil.

An endeavour was made to predict the success of induction by using a scoring system for evaluation of readiness for labour. The experience gained suggests that a scoring table of this kind may, when simplified and refined, be useful in deciding the best time for the performance of induction.

Different forms of oxytocin administration have been employed since 1965, each for one year, for induction of labour at Department I of Obstetrics and Gynaecology, Tampere Central Hospital, Tampere. In 1965 intravenous administration was used (Syntocinon Intravenous®, Sandoz), in 1966 oxytocin was given transbuccally (Syntocinon Buccal®, Sandoz) and in 1967 by intranasal application (Partocon N®, Ferring). The results have been published (7) and form an important background for the clinical study reported here. Among other authors, Borglin (1), Müller & Osler (8) and Sjöstedt (10) stressed the usefulness of a comparative method in which inductions are performed on as uniform material as possible, by different oxytocin preparations in similar conditions.

Desamino-oxytocin, which was synthetised by Du Vigneaud et al. (12), is regarded as biologically 1.5– times more active than oxytocin. On the other hand, the qualitative differences have been found to be small and in the opinion of some authors do not exist at all (3, 4, 9).

Favourable results with the clinical application of desamino-oxytocin have already been reported by e.g. Jamston (6), Urbanus (11) and Böckler (2).

MATERIAL AND METHODS

During 1968, desamino-oxytocin buccal tablets, under the name of ODA 914 (supplied by the courtesy of Sandoz), each containing 25 Human Units (H.U.) (= 17.5 International Units), were used for induction of labour. Tablets were given under the supervision of a midwife, one tablet at a time, the next one as soon as the previous one had dissolved. However, the interval was prolonged or the administration of tablets discontinued once a regular contraction pattern was established. The maximum dosage was 20 tablets, i.e., 500 H.U. (= 350 I.U.).

The induction was started early in the morning after an amnioscopy. If it failed, no new attempt was made for 48 hours. On amnioscopy special attention was paid to the ripeness of the cervix and to other factors that affect readiness for labour. A scoring table was kept for each parturient in accordance with the scoring system introduced by Fields and his co-workers in "Induction of Labor" (5). This scoring system, slightly modified, is shown in Table I.

Induction was performed on 259 parturients, of which 127 (49%) were primigravidae. Amniotomy was performed at the beginning of induction on only 3 patients; it was not performed otherwise until a regular contraction pattern had been established. In 12 cases (4.6%) premature rupture of the membranes was the main indication for induction.

The number of deliveries in 1968 was 2 160. Induction was performed on 44 parturients by methods other than ODA 914 tablets. The total of inductions was thus 303 (14%).

Induction was classified as successful if delivery took place within 48 hours of the inception of the intervention. This report deals chiefly with the experience gained from

Table I. *Result of pregnancy test (Pregnosticon®) after legal abortion*

Days post abortion	Positive	Negative	Not performed
1	3	—	—
3	30	—	2
5	—	—	10
7	13	4	15
9	4	9	19
11	3	9	20
12	1	9	2
14	0	10	—

tive tests, 2 days later 13 out of 17 cases (76%) had positive tests, and even on day 11 in 3 out of 12 cases (25%) the tests were positive. In no case was there clinical evidence to suggest that any placental tissue remained in the uterus.

DISCUSSION

The present investigation shows that about 25% of patients can have a positive pregnancy test as late as 11 days after the ovum has been evacuated. Of course it cannot be excluded with certainty that this gonadothrophin excretion is due to some placental tissue remaining in the uterus. However this seems unlikely as great care was taken in emptying the uterus, and placental tissue tends to be rather loosely attached to the uterine wall.

Another explanation could be that the pregnant female contains large amounts of chorionic gonadotrophins which are only slowly eliminated It was found by Midgley & Jaffe (5) in postpartum women and Wide et al. (9) in cases of amenorrhoea that the elimination of chorionic gonadotrophins from plasma started with a half-life time of approximately 8 hours, but Midgley & Jaffe followed their cases for several days and found that after the initial phase the half life time increased to over 30 hours. It is also well known that the urinary excretion of these hormones in human pregnancy is great and has large individual variations (4 8)

The explanation of our results may be a combination of the high and individually varying production and the slow elimination of chorionic gonadotrophins.

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EXPERIENCE OF THE USE OF DESAMINO-OXYTOCIN BUCCAL TABLETS AS A METHOD OF INDUCING LABOUR, AND OF A SCORING SYSTEM FOR EVALUATION OF READINESS FOR LABOUR

Jarmo Laine

From Department I of Obstetrics and Gynaecology (Head: Keijo Saarni), Tampere Central Hospital, Tampere, Finland

Abstract. Induction of labour was performed on 259 parturients by means of desamino-oxytocin (ODA 914) buccal tablets. The maximal dose was small (350 I.U.). Primary induction was successful in 64% of the cases. The incidence of maternal complications was small.

Neonatal asphyxia was diagnosed in 12 newborn infants (4.6%). Asphyxia was most common in those cases in which toxaemia was the indication for induction. Intra partum and neonatal mortality were nil.

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Days post abortion	Positive	Negative	Not performed
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5	22	—	10
7	13	4	15
9	4	9	19
11	3	9	20
1	1	9	22
14	0	10	22

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Table III. *Results of inductions of labour dosage of ODA*

Percentages in parentheses
I O.T = Induction Onset of Delivery Time
I D.T Induction Delivery Time

		Dose of ODA in H U					Aver I O.T (hours)	Aver I.D.T (hours)
		75–100	125–200	225–300	325–400	425–500		
Negative								
N delivery in 48 hours	93 (36)	—	1	—	—	92	—	—
Positive								
Spontaneous delivery in 48 hours	152 (59)	7	25	24	23	73	3	8
Caesarean section in 48 hours	11 (4.3)	—	—	—	1	10	1	9
Vacuum extractor in 48 hours	3 (1.2)	—	—	1	1	1	1	5
Inductions, total	259							

that t was the main indication for section. When induction failed the percentage of Caesarean sections was as high as 16. The most important reasons then were the basic indications, mostly postmaturity and pre-eclampsia, for which induction had been undertaken. Three out of 15 Caesarean sections after unsuccessful induction were performed because of *foetal distress*.

The distribution of the series into groups according to parity age and period of gestation with the corresponding success rates for induction are show in Fig. 1 The verage interval between the start of induction and the delivery (I.D T) is also given.

In this series the result was better than average if the parturient was aged 20–29 induction was done in the 39th–42nd week of pregnancy and the parity was II IV The average I.D T decreased with the increase in parity or age.

The success ratio varied considerably in the different groups. An objective comparison of the different induction methods obviously requires the collection of series of parturients which is as homogeneous as possible.

Table IV shows a comparison of the outcome of induction with the score warded to the parturient for evaluation of readiness for labour in accordance with the scoring table presented in Table I. There seems to be a good correlation between the total number of points and the success of the induction. In the group with the lowest score (≤ 8) the success rate was only 35 %. By contrast, when the score was 17–18, induction rarely failed. There was not a single operative delivery in the groups in which the score was 15 or more

Examination of the different criteria of evaluation shows that when the presenting part descends into the true pelvis (item I) and more criteria indicating the ripening of the cervix (items E, F

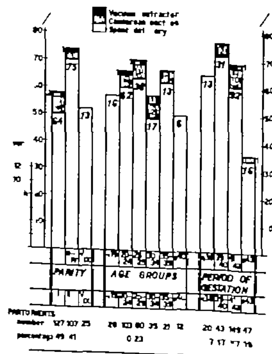


Fig. 1 Relation of parity age groups and period of gestation to percentage of successful inductions; mode method of delivery and average induction delivery time. (Absolute numbers in columns.)

Table I *Scoring system for evaluation of readiness for labour*

	score
A. *Calculated date of co finement (D C.)*	
Poor menstrual history D C. uncertain	0
More than 3 weeks prior to D C.	0
1–3 weeks prior to D C.	1
Within 1 week of D C. o later	2
B. *Patient attitude*	
Resisting or fearful	0
Hesitant	1
Compliant, enthusiastic	2
C. *Estimated size of infant*	
Less than 2 500 g	0
Uncertain but at least 2 500 g	1
Def nitely more than 2 500 g	2
D *Uterine irritability after manual palpation*	
No contraction	0
Slight contraction	1
Full, firm contraction	2
E *Softness f cervix*	
Firm	0
Somewhat softened and indentable	1
Soft and dilatable	2
F *Position of cervix: cervical canal pointing*	
Posteriorly	0
About 45 to the axis of vagina	1
Toward the introitus	2
G *Effacement of cervix*	
Internal os still present	0
Internal os effaced, length of the canal ≤ 1 cm	1
Cervical canal completely eff ced	2
H *Dilatation of external os*	
Admits only a finger or is closed	0
3 cm	1
More than 3 cm	2
I *Station of presenting part*	
Higher than − 1	0
Between 0 nd − 1	1
Lower than 0	0
J *Recent change in vaginal discharge*	
No change	0
Increase of mucous discharge	1
Blood-tinged discharge	2

the first induction. Some observations are also presented on the cases in which induction had to be attempted a second time or even more frequently

INDICATIONS FOR INDUCTION

These are given in Table II. Postmaturity was the most frequent indication for induction, followed by pre-eclampsia. Both reasons were often combined. The pregnancy was classified as postmature if 10 or more days had elapsed from the date of confinement calculated on the basis of menstruation

141 parturients (55%) were examined radiologically for maturity of the foetus. In 4 cases the foetus was estimated to be premature, 63 foetuses (46%) were regarded as mature and 52 (37%) as postmature. In 20 cases the result was uncertain. Other methods for the diagnosis of postmaturity were not used.

RESULTS AND PRELIMINARY COMMENTS

The success of th inductions is presented in Table III It gives also the dosage of ODA 914 buccal tablets and the average interval between the instigation of induction and the beginning of regular contractions and parturition itself Out of 259 inductions, 166 (64%) were successful. In 15? cases the delivery was spontaneous. Eight of the babies were delivered in breech presentation Caesarean section proved imperative in 11 cases (4 3%) and in 3 cases vacuum extraction had to be resorted to (1.2%)

Fifty-three (32%) of the successful inductions resulted in delivery within 6 hours, 128 (77%) within 12 hours and 157 (95%) within 4 hours. Less than 400 H U (i.e 280 I U) of desamino-oxytocin was required in over half of the cases.

The commonest reason for terminating an induction by Caesarean section after regular contractions had begun was foetal distress i.e., in 7 out of 11 cases. In 2 cases the cephalopelvic disproportion became so evident during induction

Table II *Indications for induction of labour*

Percentages i parenthesis

	Principal indication	Additional indication
Postmaturity	137 (53)	28
Pre-eclampsia	63 (4)	78
Premature rupture of membranes	12 (4.6)	6
Intra-uterine foetal death	1 (0 4)	—
Latent diabetes	14 (5 4)	
Tw pregnancy	(0.8)	3
Previous complications of delivery	2 (0 8)	10
Other reasons	28 (11)	54
	259	

Table III. *Results of inductions of labour dosage of ODA*

Percentages in parentheses
I O T Induction Onset of Delivery Time
I D T = Induction Delivery Time

		Dose of ODA in I U					Aver I O T (hours)	Aver I D T (hours)
		75–100	125–200	225–300	325–400	425–500		
Negative								
No delivery in 48 hours	93 (36)	—	1	—	—	92	—	—
Positive								
Spontaneous delivery in 48 hours	152 (59)	7	25	24	23	73	3	8
Caesarean section in 48 hours	11 (4 3)	—	—	—	1	10	1	9
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In this series the result was better than average if the parturient was aged 20–29 induction was done in the 39th–42nd week of pregnancy and the parity was II–IV The average I.D T decreased with the increase in parity or age.

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Table IV shows comparison of the outcome of induction with the score awarded to the parturient for evaluation of readiness for labour in accordance with the scoring table presented in Table 1 There seems to be a good correlation between the total number of points and the success of the induction. In the group with the lowest score (≤ 8) the success rate was only 35%. By contrast, when the score was 17–18 induction rarely failed. There was not single operative delivery in the groups in which the score was 15 or more.

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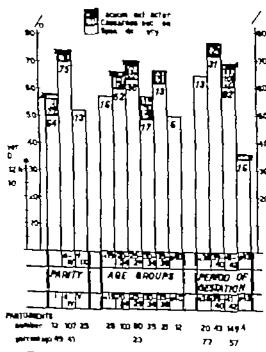


Fig. 1 Relation of parity age groups and period of gestation to percentage of successful inductions; assoc method of delivery and average induction delivery time. (Absolute numbers in columns.)

Table IV *Relation of scoring of readiness for labour to outcome of primary induction*

Percentages in parentheses

		Number of parturients	Spontaneous delivery	Operative delivery
Total score				
–8		34	12 (35)	2
9–10		68	34 (50)	3
11–12		74	43 (58)	4
13–14		56	41 (73)	5
15–16		13	10 (77)	—
17–18		14	12 (86)	—
		259	152	14
Individual score				
A.	0	7	4 (57)	—
	1	18	12 (66)	1
	2	234	136 (58)	13
B.	0	1	1	—
	1	86	47 (54)	3
	2	172	104 (60)	11
C.	0	—	—	—
	1	9	4 (44)	2
	2	250	148 (59)	12
D	0	41	19 (46)	1
	1	197	116 (59)	12
	2	21	17 (80)	1
E.	0	29	12 (41)	3
	1	165	92 (56)	7
	2	65	48 (73)	4
F	0	43	23 (53)	—
	1	161	90 (56)	12
	2	55	39 (70)	
G	0	104	51 (49)	5
	1	90	49 (54)	8
	2	65	52 (80)	1
H	0	117	53 (45)	7
	1	121	80 (65)	7
	2	1	19 (90)	—
I	0	143	74 (51)	8
	1	112	75 (67)	5
	2	4	3 (75)	1
J	0	86	44 (51)	6
	1	140	85 (61)	6
	2	33	23 (69)	

G H and I) were seen, the percentage of successful inductions grew correspondingly and operative delivery had to be resorted to rarely It is difficult to measure cervical ripeness. However routine amnioscopy prior to the start of induction has the great advantage of giving the examining obstetrician at the same time a clear picture of the cervix.

Only a small proportion of the inductions were performed before the 40th week of pregnancy (item A) Generally they were as successful as the other inductions.

There seemed also to be a favourable correlation between a positive patient attitude and the success of the intervention (item B). The differences were small, however

The readiness of the uterus to contract was evaluated only on the basis of palpation (item D) Because of the inaccuracy of the investigation method a change-over to a standard oxytocin sensitivity test must be recommended.

About 2/3 *of the first inductions were performed* on parturients whose readiness score was less than 13 The intervention failed in about half of these patients. Elective inductions would have been avoided if possible but were justified because of anxiety about the maternal and especially the foetal prognosis, which forced action without delay The more frequently early inductions can be avoided the better This can be achieved for example by the prophylaxis and treatment of toxaemia and the more reliable diagnosis of postmaturity and foetal intra-uterine asphyxia. A more favourable point of time for delivery can then be awaited with greater confidence.

Cases with premature rupture of the membranes in this group totalled 18 The first induction resulted in delivery in about 80% of them.

Maternal complications are seen in Table V It must be noted that in 16 cases (6.2%) frequent and or violent contractions developed. As to the newborn in these cases, 2 were asphyxiated at 1 minute (Apgar score 5–6) and one at 5 minutes (Apgar score 6)

In 4 parturients uterine exhaustion developed during the induction. In 2 cases it became an indication for the use of vacuum extractor None of the newborn was asphyxiated

Table V *Maternal complications*

Percentages in parentheses

Uterine exhaustion		4 (1.5)
Uterine hypertonus		16 (6.2)
Retained placenta		1 (0.4)
Atony of uterus post partum		3 (1.2)
Postpartum haemorrhage	500–800 g	13 (5.0)
	more than 800 g	5 (1.9)
Prolonged labour		6 (2.3)
Vaginal tear		6 (2.3)
Third degree perineal laceration		1 (0.4)
Lochiometra		23 (8.9)
Puerperal endometritis		(0.8)

ble VI. *Relation of amniotic fluid, size of placenta, umbilical cord and foetal heart to Apgar score ociated principal indication for induction*

rcentages in parentheses

		Apgar score at 1 min < 6	Apgar score at 5 min < 6	Principal indication for induction		
				Post-maturity	Pre-eclampsia	Other
mniotic fluid						
f normal colour	217 (84)	7 (3.2)	2 (0.9)	116 (54)	47 (22)	54 (25)
ontaining meconium	42 (16)	5 (12)	2 (4.8)	21 (50)	16 (38)	5 (12)
	259					
ize of placenta						
< 500 g	72 (28)	7 (9.8)	3 (4.2)	31 (43)	21 (29)	20 (28)
01–600 g	97 (37)	2 (2.1)	1 (1.0)	56 (58)	25 (26)	16 (16)
600 g	90 (35)	3 (3.3)	—	50 (56)	17 (19)	23 (26)
	259					
Umbilical cord						
Normal	164 (63)	5 (3.0)	2 (1.2)	87 (53)	38 (23)	36 (22)
Around the neck and/or the body	97 (37)	7 (7.2)	2 (2.1)	49 (51)	25 (26)	23 (23)
Prolapse	1 (0.4)	—	—	1	—	—
	264					
Foetal heart rate						
Normal	237 (90)	8 (3.4)	3 (1.3)	126 (54)	52 (22)	54 (23)
110/min and/or > 160 /min	20 (7.6)	4 (20)	1 (5.0)	6 (30)	9 (45)	5 (25)
Irregular but within normal range	7 (2.7)	—	—	5 (72)	2 (28)	—
	264					

Immediately after the baby's birth the mothers were given an intramuscular injection of 1 ml of Syntometrin®. In one case the placenta had to be removed manually. Postpartum haemorrhage was slightly more common than in inductions performed in 1964–1967 in this department (7).

Intrapartum and neonatal mortality was nil. At 1 minute 10 of the newborn (3.8%) were moderately asphyxiated, with an Apgar score of 3–6. In addition, 2 were severely asphyxiated, with an Apgar score of 1–2, one of them had pneumothorax. At 5 minutes, only 4 (1.5%) were moderately and none severely asphyxiated.

In spite of a normal Apgar score, signs of postnatal asphyxia developed later in 5 newborn, and in further 4 cases aspiration syndrome was diagnosed. All recovered well. Other complications of the newborn were negligible.

It seems to be highly significant that in 8 of the 12 cases of neonatal asphyxia (4.6%) at 1 minute toxaemia had been the principal indication for the induction, and postmaturity in 3 cases. In all 4 cases in which asphyxia persisted at 5 minutes the induction had been performed because of toxaemia.

The relative proportion of asphyxia increased in those cases in which the child was not born until after two or more inductions (vide infra). As the outcome of primary induction 171 babies were born and two of them (1.2%) were severely and four (2.3%) moderately asphyxiated.

Relation of amniotic fluid, size of placenta, umbilical cord and foetal heart rate to Apgar score, and associated principal indication for induction are shown in Table VI. In 42 cases where the amniotic fluid contained meconium, 12% of the newborn were asphyxiated at 1 minute and 4.8% at 5 minutes, i.e. about four times more often than if the liquor was clear. The role of pre-eclampsia as the principal indication for the induction was more common in the meconium group: 33 versus 22%.

Similar conclusions can be made as to the relation of the size of the placenta to these factors although the differences are not very marked. Neonatal asphyxia and the role of toxaemia as an indication for the induction were more common, when the weight of the placenta was less than 500 g.

Asphyxia was expectedly more common in the

Table VII. *Results of secondary inductions*

Percentages in parentheses

Failures after the first induction		93		
Spontaneous delivery before further inductions		5 (5 4)		
Number of secondary inductions		88 (95)		

	II *Induction*	III *Induction*	IV *Induction*	*Total*
Number of cases	88 (95)	35 (38)	1 (13)	
SIV[a]/ODA	6/62	19/16	7/5	
Within 48 hours				
Spontaneous delivery	36 (41)	18 (52)	8 (67)	62 (67)
Caesarean section	8 (8..)	(5 7)	3 (25)	13 (14)
Vacuum extractor	1 (1 1)	1 (2.9)	—	(2.2)
	45 (51)	1 (60)	11 (9)	77 (83)
After 48 hours				
Spontaneous delivery	5 (5 7)	2 (5 7)	1 (8 4)	8 (8.6)
Caesarean section	(2.3)	—	—	2 (2..)
Vacuum extractor	1 (1 1)	—	—	1 (1 1)
	8 (8..)	(5.7)	1 (8.4)	11 (1)
Left for III induction	35			
Left for IV induction		1		

SIV = Syntocinon® intravenous.

newborn with coiling of the cord around the neck and or the trunk.

An abnormal foetal heart rate was noticed in connection with 20 inductions. Half of these parturients had toxaemia and 4 of the newborn were asphyxiated at 1 minute On the other hand, the heart rate was regarded as normal in 8 cases in which the newborn was distinctly asphyxiated at 1 minute. It is obvious that following the heart rate with a stethoscope alone does not give sufficiently accurate information on the foetal status.

A microblood sample was taken from the head of the foetus in 7 cases in which intra-uterine asphyxia was suspected. However the pH values were normal and none of these newborn was found to suffer from neonatal asphyxia.

The results of secondary inductions are shown in Table VII. The first induction failed in 93 cases. Five of these parturients delivered spontaneously later on, a second attempt was made on 88, a third on 35 and a fourth attempt at induction on 12. In addition to ODA, Syntocinon® was given in an intravenous infusion (5 I U in 500 ml of 5% glucose solution) relatively more often as the number of attempts increased. The success ratio was low At the second attempt spontaneous delivery resulted in 41 at the third in 52 and at the fourth in 67% Operative procedures had to be performed frequently The results achieved reveal that readiness for labour had been assessed initially as poor in these cases: the sum of the readiness score for 72 (84%) of the group of 93 parturients was smaller than 13

In 10 (11%) out of 88 second inductions uterine exhaustion was diagnosed. The same complication was noted in five of 35 third attempts and in two of 12 fourth inductions. Violent and/or frequent contractions occurred in two of 35 third inductions, and in two of 12 fourth inductions.

As already stated, asphyxia was relatively more frequent in conjunction with secondary inductions. Four (7 6 %) of the 53 infants born as the result of secondary induction were moderately asphyxiated (Apgar score 3–6) Thirty-five babies were delivered as the result of third and fourth inductions and 2 (5 7%) of them were moderately asphyxiated.

DISCUSSION

ODA 914 buccal tablets achieved in the clinical experiment conducted by the present author roughly the same results in the induction of labour as earlier oxytocin preparations which have already gained extensive use The success rate was somewhat smaller (64%) in primary induction than the results achieved earlier with intravenous Syntocinon® infusion (67) Syntocinon® buccal tablets (75) or Partocon IN® intranasal solution (79) in this department (7)

The maximal ODA 914 dose per induction was very small, only 350 I U., and often only 100–.80 I.U was necessary With the Syntocinon® buccal tablets used previously the ordinary dose was 1 700–1 900 I.U and the maximal dose .600 I U Even if desamino-oxytocin is biologically roughly twice as active as oxytocin, the ODA 914 dosage was appreciably low In addition individual doses were kept the same in this series, but a progressively increasing dosage would obviously

have been more effective. The author feels that in selected cases the total dosage of ODA 914 could be 700–1 000 I U per induction without increasing the risk of complications.

Caesarean section was performed on approximately 10% of the women. The indication was suspicion of foetal distress in almost 40% of the cases. On the other hand, there was neither intra partum nor neonatal mortality. The incidence of neonatal asphyxia was also relatively small, the Apgar score of 12 (4.6%) infants at 1 minute was six or less and at 5 minutes there were only 4 children in this category (1.5%). Asphyxia occurred slightly less frequently in this series compared with the inductions undertaken in 1964–1967. When asphyxia was established, toxaemia had been in relative terms clearly the more frequent main indication for induction (see above). Waiting for spontaneous delivery would obviously only have impaired the prognosis for the baby in these cases. The benefit of induction compared with Caesarean section is, however, difficult to prove in individual cases.

There is reason to point out that the success of induction was considerably influenced in the present series by the patient's readiness for labour (especially part criteria indicative of the "ripeness of the cervix), her age, parity and also the duration of the pregnancy. Objective comparison between the various methods of induction and oxytocic agents is difficult to make unless the material is highly homogeneous in these respects.

The use of oxytocin preparations increases the risk of hypertonic contractions. These occurred in 6 % of this series on the basis of clinical observations. Contractions were restored to the normal level by temporary interruption of induction and or other therapeutic procedures.

The other maternal complications were minor and did not differ significantly from the complications of the normal material in the department.

The percentage of primary inductions in the total of deliveries in the department has more than doubled in 5 years. It was 6.5 in 1964 and 14 in 1968. The indications have thus not been equally strict, although purely elective inductions have not been performed. The number of inductions has been increased especially by the fact that even very mild toxaemia has been accepted as a suitable indication. Previously spontaneous onset of labour was often awaited in such a case. In 1966, when Syntocinon® buccal tablets were used, toxaemia accounted for 30% of all the indications and in 1968 in the present series for 55%.

In this study of different oxytocin preparations, each for one year it was unfortunately not possible, owing to the nature of the clinical work, to use only one preparation for all inductions in the course of the year. Intravenous Syntocinon® infusion was often used in earlier years in the cases in which the mother's and/or infant's prognosis was estimated to be subaverage. This method was partially abandoned later.

The results for the use of different oxytocin preparations are not directly comparable for the reasons mentioned above. If elective inductions were performed on healthy parturients the double blind technique could obviously also be applied. It would then be possible to compare more reliably the different oxytocin preparations, provided that the readiness of the different parturient groups for labour was also homogeneous.

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INFLUENCE OF VAGINAL FLORA ON BODY TEMPERATURE DURING THE PUERPERIUM

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Abstract. Vaginal smears from 342 pregnant women were studied 4 weeks before expected term.

70 patients were subsequently excluded because of Caesarean section, manual removal of the placenta, or fever caused by extragenital infection during the puerperium. The smears were classified as suggested by Wied. 56% showed no infection (A), 14% showed predominance of *Bacillus vaginalis Döderlein* (B), 7% showed cocci (C), 11% showed *Trichomonas vaginalis* (D), and in 12% mixed infection was found (E).

The non-infected (A+B) and the infected (C+D+E) patients were divided into 4 groups each, according to whether more or less than 12 hours had elapsed from rupture of the membranes to delivery and whether [illegible] or 3 pelvic examinations had been performed after rupture of the membranes. The mean temperature graph for the "infected" and the "non-infected" patients in the various groups was compared, but no significant differences were demonstrated.

Nine patients developed puerperal fever [illegible] rectal evening temperature exceeding 38°C, after the first day which could not be ascribed to an extragenital focus. Four of these patients had normal vaginal flora antenatally, while five had evidence of antenatal infection. On statistical calculation, however, puerperal fever was not significantly more common among patients with infected than in those with non-infected vaginal smears.

Two infants developed fever after birth. Both mothers had non-infected vaginal smears.

The purpose of the study was to investigate whether patients with pathogenic vaginal flora, as detected antenatally by cytological examination of vaginal smears, are more prone to puerperal pyrexia than are patients with normal flora. In this analysis regard was paid to the number of pelvic examinations which had been performed and to the time which had elapsed from the rupture of the membranes to delivery. No regard was paid to whether or not the patients had received antibiotics.

The normal puerperium is afebrile. However, the temperature may rise within the first 24 hours post partum as a result of the increased muscular activity. Thereafter the temperature record shows the normal daily diphasic course.

Trolle (5) defines puerperal fever as a rectal evening temperature exceeding 38 C exclusive of the evening of the first day which cannot be ascribed to an extragenital cause.

Probably the majority of cases of puerperal fever are caused by exogenous factors, the infection being due to contamination from the surroundings. Apart from this, it is reasonable to believe that some infections are of endogenous origin, from pre-existing bacteria in the vagina, since the uterine cavity invariably contains bacteria of vaginal origin from the second day post partum. Factors such as haemorrhage, prolonged labour, Caesarean section, and retained portions of the placenta predispose to the occurrence of puerperal fever (1, 3). The reasons why bacterial invasion does not always result in puerperal fever are diverse: First, if labour has not been prolonged the bacteria do not reach the uterus until the second day. At this stage a protective wall of granulations has formed in the endometrium. Secondly the constant flow of lochia flushes out a large number of bacteria. Thirdly most of the bacteria which do reach the endometrium are of low virulence (3).

PREVIOUS INVESTIGATIONS

At least two papers have been published recently concerning the special relationship between fever

Table I *Frequency of different bacteriological types of smear from 472 patients during pregnancy*

Type of smear	Frequency *n*	%
A. No infection	264	56
B. Bacillus vaginalis Döderlein	66	14
C. Cocci or streptococci	35	7
D Trichomonas vaginalis	52	11
E. Mi ed flora	55	12
Total	472	100

during the puerperal period and pathogenic vaginal flora.

In Houdek, Král & Pelák's (4) study of 109 patients with putrid bacterial infection and Trichomonas vaginitis—demonstrated by culture and microscopy—62 (56%) showed an elevation of temperature during the puerperal period. However these authors define fever as a temperature exceeding 37 4 C on the second and fifth day post partum. They recommend examination of vaginal smears before labour and treatment in cases where there is evidence of infection.

Faver (2) also recommends treatment if pathogenic vaginal flora is present prior to labour This author studied 2 groups of 100 patients. In group (*a*) all the patients with cytological evidence of vaginal infection (Papanicolaou 3–4) were treated, while those in group (*b*) were not. In group (*a*) 3 patients developed endometritis (temperature exceeding 37.5 C) and 2 infants developed pyoderma. In group (*b*) 9 patients developed puerperal endometritis and 6 infants pyoderma.

PRESENT INVESTIGATIONS

Vaginal smears were taken from 542 patients at out patient antenatal visits 4 weeks prior to term The specimens were obtained with a glass pipette from the lateral vaginal wall in the proximal one-third of the vagina. The secretion was smeared on slides and fixed and stained by the method of *Harris Shorr 70 of the 542 patients* were subsequently excluded from the study because 30 had Caesarean section, 12 had manual removal of the placenta, 9 developed fever due to extragenital infection during the puerperium, and 19 could not be checked.

According to Wied (7) the smears may be divided into the following groups

(A) Smears in which the vaginal flora did not cause changes influencing the assessment. In pregnant patients these smears are characterized by intermediate cells, often of the navicular type

(B) Smears with a predominance of Döderlein bacillus. In these smears the cells are characterized by cytolysis, with a large number of free nuclear remnants.

(C) Smears characteristic of the coccus or streptococcus type. In these smears the intermediate cells lie as islets in a turbid ground substance rich in bacteria.

(D) Smears infected with *Trichomonas vaginalis* The organisms are seen as piriform bodies with a delicate prickled pattern at one end of the cytoplasm. Frequently there is a perinuclear halo. In a number of cases with recent infection pseudo-acidophilia of the intermediate cells is also seen.

(E) Smears showing mixed infection. These smears present a polymorphous appearance, but often with a considerable content of leukocytes and varying degrees of autolysis of the intermediate cells.

Fungal infections are not classified separately as the mycelia are often lost in the course of the preparation

According to this classification groups A and B are considered non-infected and groups C, D and E infected

The vaginal smears from the 472 patients were as shown in Table I.

Table II

	N of patients	*Infected* vaginal smear	≥3 pelvic examinations after *rupture of* the membranes	12 hours from rupture of the *membranes* until delivery
Group 1	271	–	–	–
Group 2	115		–	–
Group 3	35	–		
Gro p 4	16			
G oup 5	6	–	–	
G oup 6	5			+
G oup 7	19			
Group 8	5			
Total	472			

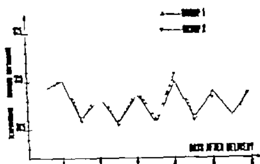


Fig. 1. Mean daily temperature graph during the first 6 puerperal days for the patients of groups 1 and 2 (Table II)

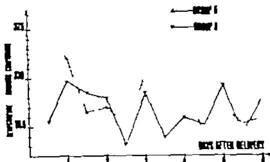


Fig. 3. Mean daily temperature graph during the first 6 puerperal days for the patients of groups 5 and 6 (Table II)

I each individual the time from the rupture of the membranes until delivery and the number of pelvic examinations after rupture of the membranes were recorded. The patients were classified in 8 groups, based on

(I) whether or not the vaginal smears showed evidence of infection,

(II) whether fewer than 3 or more than 3 aginal examinations had been performed,

(III) whether the membranes had been ruptured for less or more than 12 hours prior to deli ery (cf. Table II).

For each group the mean temperature on each of the first 6 puerperal day was calculated. In Figs. 1–4 comparison of these findi gs is made between groups 1 and 2, between groups 3 and 4 between groups 5 and 6, and between groups 7 and 8.

Nine of the 472 patients developed puerperal feve . Their distribution by type of vaginal smear is shown in Table III.

It is reasonable to believe that the bacterial flora of the newborn infant is derived from the birth canal (6). The infants were, therefore, examined for febrile diseases. One infant had pulmonary atelectasis and pneumonia and another had pemphigus neonatorum. In both mothers the vaginal smears had been non-infected, and only the mother of the infant with pemphigus had had more than 3 pelvic examinations after the membranes had ruptured.

DISCUSSION

As is apparent from Figs. 1–4 there was no difference in mean temperature level between the groups compared. Group 8 (Fig. 4) did show a somewhat higher mean temperature than group 7 but no significance can be attached to this, as only small umbers of patients were involved.

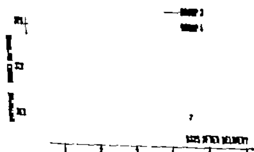


Fig. Mean daily temperature graph during the first 6 puerperal days for the patients of groups 3 and 4 (Table II)

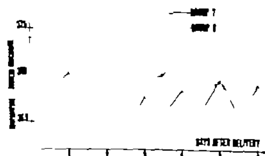


Fig. 4. Mean daily temperature graph during the first 6 puerperal days for the patients of groups 7 and 8 (Table II)

Table III. *Puerperal fever among 472 patients by bacteriological type of smear*

Type of smear	No of patients with puerperal fever
A. No infection	4
B. Bacillus vaginalis Doderlein	0
C. Cocci or streptococci	1
D. Trichomonas vaginalis	1
E. Mixed flora	3
Total	9

The mean temperature pattern for a large number of patients must be considered a good method for evaluating a general trend, whereas fever in a few patients has little effect on the mean values Therefore patients with puerperal fever were analysed separately (Table III)

142 of the total of 472 patients were non infected. It will be seen that the tendency to infection in both the infected and in the non-infected group is slight. In fact, there is a pre ponderance of patients with puerperal fever in the infected group but the numbers are too small to demonstrate any statistically significant dif ference, $\chi^2 = 1\ 73$ and $p < 0\ 1$

On the basis of the present findings puerperal fever or an elevated temperature curve does not seem to be more frequent in patients with in-fected than in patients with a non infected vagina.

The results indicate that the number of pelvic examinations done after the rupture of the mem-branes has had considerably more effect than the primary flora upon the course of the tempera ture

In the present material an infected vaginal flora antenatally did not predispose to febrile disorders among the infants.

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WEIGHT LOSS IN SECONDARY AMENORRHEA

A Gynaecologic Endocrinologic and Psychiatric Investigation of 54 Consecutive Clinic Cases

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Abstract. Fifty-four consecutive cases of secondary amenorrhea seen in the Department of Obstetrics and Gynaecology of Umeå University Hospital were systematically studied from the somatic, endocrinologic and psychiatric points of view. The study showed that 51 patients had lost weight at or about the time of onset of amenorrhea and that 41 of these had symptoms characteristic of anorexia nervosa; some were cachectic. In none of these cases was there any reason to suspect a primary endocrine disturbance. On the other hand, two of the patients who had not lost weight, had significantly elevated urinary 17-ketosteroids in both total and fractional analyses.

The connection between starvation, psychogenic amenorrhea and anorexia nervosa is discussed.

Many of the patients who come to a gynaecological clinic because of secondary amenorrhea state that they have had nervous symptoms and/or show clear signs of psychic insufficiency at the time of examination. This has given rise to the concept of psychogenic amenorrhea (see Reifenstein, 1946). According to Schwartz (1963) such amenorrhea may be caused by psychic traumata and conflicts and may also be present in anorexia nervosa and grossesse nerveuse, as well as in patients with neurotic character disturbances.

Duret-Cosyns (1969) presented 12 patients with amenorrhea, all under 25 years of age, who were referred from the gynaecological clinic to the department of psychosomatic medicine of Hospital Saint Pierre in Brussels, where they were examined. The patients were not described in detail. The investigation showed that all the patients, at the time they became amenorrheic or shortly before, had lost weight as a result of anorexia, vomiting or dieting. Since it is known that prolonged or intensive starvation can give rise, not only to amenorrhea, but also to mental symptoms (Helweg-Larsen et al., 1952, Brozek, 1953, Sletten et al., 1967, Lawlor & Wells, 1969, Swanson & Dinello, 1970) the question arises whether so-called psychogenic amenorrhea can be related to starvation or to a deficient diet. In order to answer this question, a number of cases of secondary amenorrhea have been studied.

MATERIAL AND METHODS

The material consisted of all 54 patients who were seen in the Gynaecological Clinic of Umeå University Hospital during the years 1969 and 1970 with a diagnosis of secondary amenorrhea. (This diagnosis was made only if the amenorrhea had persisted for at least 6 months.) The number of patients was 54, 50 of whom were 25 years of age or younger at the time of the investigation; the average age was 22 years (range 16–37 years). Ten patients were married at the time of investigation. One woman had had one child, and two had had two children; the others were childless. Fourteen of the patients had had gymnasium education, held an academic degree or were engaged in an occupation that required this level of education; 24 had had the equivalent of high school or vocational school training or were engaged in an occupation requiring this level of education; 16 patients had had only an elementary school education or were engaged in an occupation that did not require more extensive formal education.

In addition to general status, with recording of height and weight, the physical examination included the usual routine gynaecological examination and, in addition, assays of gonadotropic hormones, fractional 17-ketosteroids, 17-OH-steroids and, in some cases, oestrogens. Metabolic tests were also carried out: basal metabolism, protein-bound iodine and, in some cases, also iodine tracer studies. Examination of cytological specimens, usually macro-curettage, investigation of the crystallization of cervical mucus, roentgenographic examination of the sella turcica and determination of the range of vision were also in-

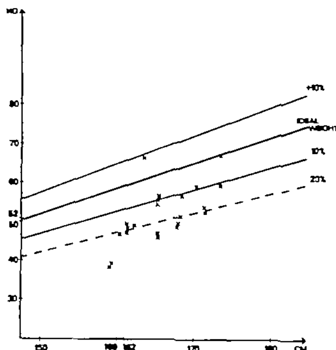


Fig 1 The patients weight in relation to height, according to Natvig.

cluded in the programme (For analysis of oestrogens and 17 ketosteroids, we are indebted to the Hormone Laboratory Sabbatsberg Hospital, Stockholm (chief Docent Mirjam Furuhjelm, M.D.).

The psychiatric investigation included a socio-psychiatric anamnesis according to standardized scheme. The questions concerned the patient heredity social conditions, previous state of physical and mental health, personality characteristics and adjustment at home at school and in the community A dietary anamnesis was also regularly taken, and an attempt was made to determine how much physical activity the patient engaged in. *While the anamnesis was being taken, the patient's mental* status was evaluated and recorded. If the patient had previously sought medical aid or had been admitted to th. hospital, the hospital record was reviewed for complementary information. The patient mental status was evaluated without our knowing the results of the somatic investigation. All psychiatric evaluations w re made *personally by the one of us who is trained in psychiatry* (I N).

The term anorexia nervosa is used in the text below according to Tolstrup definition (1957). syndrome the predominant symptom f which is anorexia of such degree f severity that it is attended, n the long run, by considerable loss of weight It is not symptom of any kno n primary *somatic illness or psychoses* and is followed by symptoms f endocrine disturbance (most commonly amenorrhea), egetative disturbances, symptoms referable to the digesti tract and neurotic symptoms

RESULTS

Some of the *findings* of the *investigation* were as follows: All but 3 of the patients, or 94 stated that weight loss was related to the onset of amenorrhea 36 patients were certain that the weight loss had preceded the onset of amenorrhea, 4 stated that the opposite was true, and the remaining 11 patients were *uncertain* as to the time sequence

The average loss of weight was about 11 kg (range 3–28 kg) *All* of the patients who reported little weight loss (less than 5 kg) were underweight at the time of examination.

The patients could be classified, according to anamnesis, in the following way

1 Those *who stated that they had lost weight* through dieting. 25 cases.
2. Those who stated that they had lost weight in connection with psychic insufficiency *10* cases.
3 Those who stated that they had lost weight in connection with a physical illness: 6 cases.
4 Those who stated that they had lost weight as a result of an inadequate diet: 4 cases.
5 Those who stated that they had lost weight as a result of excessive physical activity ? cases.
6. Those whose weight loss was of unknown origin. 4 cases.
7 Those who had not lost weight. 3 cases.

Measurements showed that most of the patients were poorly nourished Their weights in relation to their respective heights (according to Natvig) are shown in the accompanying figure It appears *from the figure that many of the patients were extremely thin, and that nearly half* were more than 20% below the ideal weight for their height

The patients somatic-gynaecologic status as unremarkable The results of the determinations of basal metabolic rate and hormone titrations are given in the accompanying table which also includes certain anamnestic data. The material has been tabulated according to the patients own statements regarding weight loss (see above)

The table shows that there were no somatic differences between the groups, if one excludes group 7 (the 3 patients who had not lost weight). Most of the patients had a low basal metabolic rate, but other tests of thyroid function and the results of iodine tracer studies were normal. The excretion of gonadotropic hormones was low or *normal, a finding which probably mainly reflects* the uncertain value of isolated hormone analyses.

Table I *Certain anamnestic data and results of investigations. The material is divided according to reports of weight loss (see text)*

Group	1 = 25	2 = 10	3 6	4 = 4	5 2	6 = 4	7 3
Reported weight loss in kg Σ	14.0	8.8	8.5	7.5	4.0	8.5	—
Had used oral contraceptives	10	3	3	2	0	1	1
Previous periods of amenorrhea (at least 2)		2	0	1	0	0	2
Picture of anorexia nervosa	4	7	2	4	2	2	0
Low BMR (borderline 15%)	20	6	3	2	1	3	0
Low total gonadotropins (<1.0 mg HMG-standard)	17	10	3	4	1	1	1

Most of the patients had low oestrogen levels; in only a few cases was the oestrogen level within the accepted limits. Excretion of 17-ketosteroids was within normal limits in about half the patients, in the others it was below the lower normal limit (see Carlström et al., 1969).

It was of special interest that in group 7, the group in which there had been no weight loss, two patients had clearly elevated 17-ketosteroid levels in both total and fractional analysis. These patients were referred for further studies, adrenal cortical hyperplasia or tumour being suspected.

As is further shown in the table, in all groups but one, the onset of amenorrhea had occurred in about 50% in connection with discontinuation of the use of oral contraceptives.

All of the 25 patients in *group 1* who reported that they had dieted, had believed themselves to be fat. The average reported weight loss was 14 kg (range 4–25 kg). All the patients in this group stated that they had grown up in a calm and secure family environment. The father of one patient had committed suicide, but this had occurred when the patient was 2 years old, the mother had married again and lived in a stable marriage during the greater part of the time the patient was growing up. There was no history of mental illness in any of the families, nor was there any history *of* metabolic disease or endocrine disorder. One or the other of the parents was said to have been "fat" in 4 cases (2 mothers and 2 fathers), otherwise there was no familial history of weight problems. All of the patients in the group stated that before they began to diet they had been normally developed, physically and mentally well and socially well-adjusted. After dieting, 23 of the 25 patients had developed symptoms characteristic of anorexia nervosa. Two of these patients were later admitted, cachectic and in poor condition, to the department of child psychiatry of Umeå University Hospital. One of them was moribund at this time and was referred to the intensive treatment unit because of incipient heart failure.

At the time of examination, 8 of the patients were judged to be suffering from psychic insufficiency. (The symptoms were mainly depressive in nature.)

The 10 patients in *group 2* who stated that they had lost weight in connection with psychic insufficiency had lost an average of 10 kg (range 3–28 kg). Eight of these patients had grown up in, or lived in, an unstable home situation. One mother had been completely incapacitated for 10 years by severe anxiety-compulsive neurosis, for which she had been treated in a mental hospital; one mother had been treated in the psychiatric clinic for prolonged anxiety symptoms; one mother was a chronic alcoholic and had been cared for at times in a mental hospital, and the father in the same family suffered from chronic psychic insufficiency and was in a mental hospital. Another father was a severe alcoholic, which had led to the parents' divorce. In 3 cases the father had died early in the patient's life and the patient had grown up with a single mother. Finally one patient had left her parental home at an early age and had been *living with* a narcotic addict (user of central-stimulating drugs) for 3 years. She had also used narcotics during the past year. All the

patients in this group reported that they had had pronounced nervous difficulties long before they began to lose weight. Eight had developed symptoms characteristic of anorexia nervosa, and one of these had been admitted to the Department of Child Psychiatry of Umeå University Hospital for treatment. At the time of examination, all 10 patients were judged to be suffering from psychic insufficiency (In four the symptoms were mainly depressive in nature six had symptoms of anxiety neurosis.)

In *group 3* in which the patients reported that they had lost weight in connection with physical illness (gastric ulcer in 1 case gastritis in 3 cases, cholecystitis in 1 case prolonged and complicated mammariplastic operations in 1 case) the average weight loss was 8.5 kg (range 6–11 kg) All the patients had grown up in complete homes and under secure conditions. None of their close relatives had had any known mental disease metabolic disease or endocrine disturbance. Two of the 6 patients described symptoms characteristic of anorexia nervosa. At the time of investigation, 3 of the patients were judged to be suffering from psychic insufficiency with predominantly depressive symptoms.

Group 4 consisted of 4 patients who all gave a history of highly unsatisfactory dietary habits (regularly missed breakfast and/or lunch) and described symptoms of the anorexia nervosa type. They had lost an average of 7.5 kg (range 3–17 kg) All 4 patients had grown up under stable and socially secure home conditions, and no mental illness, metabolic disease or endocrine disturbance was reported among the close relatives of any of the patients.

In *group 5* two girls said that they had lost weight (3 and 5 kg respectively) in connection with exaggerated physical training (daily running 5 km plus "strength training plus jazz ballet) Both patients presented a picture characteristic of anorexia nervosa. There was no known hereditary predisposition to mental illness, metabolic disease or endocrine disturbance In both cases the home environment was stable and secure

Four patients in *group 6* reported weight loss of unknown origin The average weight loss reported was 8.5 kg (range 4–13 kg) Two described symptoms of the anorexia nervosa type The family histories and home conditions were unremarkable At the time of examination, all were judged to be suffering from psychic insufficiency (The predominant diagnosis was anxiety neurosis in all cases.)

Group 7 consisted of 3 patients who stated that they had not lost weight. None had symptoms of the anorexia nervosa type A 24-year-old factory worker had had an approximately 8-month period of amenorrhea after using ovulation inhibitors. She was of normal weight for her height, and her physical and mental status were unremarkable. A 28-year-old net weaver reported that she "had always had trouble keeping weight down and had gained a great deal of weight during recent years. At the time of examination she weighed 6 kg compared with 62 kg a few years previously (height 169 cm) She had always had irregular menses of the type 6 days/5–12 weeks. Her mental state at the time of examination was unremarkable. A third patient, a 22 year-old housewife with amenorrhea of 6 months duration, had had a severe reactive depression a few years previously in connection with a brother's death in a traffic accident During the following period she had taken large doses of psychopharmaceutical agents and was still deeply depressed at the time of examination. Her weight was normal for her height and she was physically well.

DISCUSSION

As the name implies, psychogenic amenorrhea is regarded as a basically emotional disturbance It is supposed that emotional conflicts—via the adrenal cortex or the hypothalamus-hypophysis—give rise to somatic symptoms. The general nature of and clinical similarities between psychogenic amenorrhea and anorexia nervosa are striking. Mental symptoms and amenorrhea are features of both syndromes. Many patients with anorexia nervosa seek medical aid primarily for amenorrhea (Berkman, 1948 Sours, 1968 Dally 1969 Theander 1970) If it is true that anorexia and weight loss occur in psychogenic amenorrhea, which was stated by Duret-Cosyns, psychogenic amenorrhea and nervous anorexia could be parts of the same syndrome

Since Duret-Cosyns patient material was small and selected, the results of his investigation are not convincing. We therefore wished to test the generality of his results by systematically investigating a number of consecutive cases of

econdary amenorrhea seen at the Gynaecology Clinic of Umeå University Hospital from a somatic-endocrinologic-psychiatric point of view This clinic is the only one of its kind in the district, and the material was therefore judged to be representative of patients who seek medical aid for secondary amenorrhea.

At the time of examination, 51 of the 54 patients reported that they had lost weight at the time of onset of amenorrhea, in 36 cases the patients ere certain that weight loss had preceded amenorrhea. The average reported weight loss was about 11 kg. The patients who reported a weight loss less than 5 kg were all underweight. Forty one patients described symptoms characteristic of anorexia nervosa.

Most of the patients proved to be considerably below the ideal weight for their height, and the somatic-endocrinologic investigation provided no evidence that endocrine disturbance or metabolic disease as the primary cause of the amenorrhea. In only 2 cases were the hormone titration values clearly pathological, both patients being among those ho reported that they had not lost weight. Twenty-seven of the 54 patients were judged to be suffering from psychic insufficiency at the time of examination.

Anamnestic information about anorexia, weight loss, amenorrhea and mental symptoms, as well as the finding of underweight, in some cases cachexia, at the time of examination are thus in complete agreement with Duret-Cosyns' findings. Duret-Cosyns, however believed that both the mental and physical symptoms accompanying amenorrhea are secondary to emotional disturbance, as most authors believe to be the case with regard to nervous anorexia (Tolstrup, 1957 Dally 1969· Theander 1970) In f vour of this interpretation, it has been stated that these patients often have history of disturbed contact with one or both parents, that amenorrhea can appear early in the course of the disease and before the onset of weight loss, and that the mental symptoms are not of the type that are usually seen after starv tion.

However it is clear that earlier studies of anorexia nervosa have been based on highly selected patient materials. The present study alone show that only minority of the cases of anorexia nervosa usually are diagnosed (3 of 41 cases) 1 3 cases included in the present study the [illegible] syndrome had not been previously diagnosed in spite of the fact that the patients had anorexia, marked loss of weight, amenorrhea, slow pulse, low basal metabolic rate etc. Psychiatrists had instead judged them to be suffering from endogenous depression (1 case) and character neurosis (2 cases) Furthermore, the cases that have previously been described by psychiatrists have not only been severe and of long standing, but have been studied "only from a strictly intrapsychic point of iew" (Minuchin, 1970)

We thus believe that the general opinion among psychiatrists that psychogenic amenorrhea and anorexia nervosa are aspects of a primarily psychic disease is entirely too vague and poorly documented. The results of this investigation show that many patients with secondary amenorrhea ha e symptoms of anorexia nervosa and are underweight, without presenting a picture of psychic insufficiency Although the data on anorexia nervosa presented here are partly anamnestic and thus subject to some uncertainty the material in fact contained patients in a state of cachexia, and most of the patients were considerably below the ideal weight for their height. Most of these patients had dieted intensively for a long time. Others had lost weight for other reasons. Since starvation can produce both mental and physical symptoms of the type in question, w are inclined to believe that starvation might be the primary cause, and that the mental and somatic symtoms were secondary Crisp (1970) also maintains that "The trigger to the disorder is dieting behavior This hypothesis is further fa oured by the fact that the syndrome characterized by psychogenic amenorrhea and anorexia nervosa differs from other states of psychic insufficiency in that the patients often lack a history of hereditary predisposition to mental illness and/or of disturbed environmental circumstances (Theander 1970) and in that the symptoms can become milder or even disappear entirely if the patient's nutritional state can be improved (Hurst, 1939· Brozek, 1953 Frahm, 1969· own observ tions). The claim that the mental symptoms associated with starvation are of a type other than those seen in connection with psychogenic amenorrhea and anorexia nervosa, may very well depend on differences in the type and extent of weight loss. As the patient' mental and physical state deteriorates, the capacity to respond with

patients in this group reported that they had had pronounced nervous difficulties long before they began to lose weight. Eight had developed symptoms characteristic of anorexia nervosa, and one of these had been admitted to the Department of Child Psychiatry of Umeå University Hospital for treatment. At the time of examination, all 10 patients were judged to be suffering from psychic insufficiency (In four the symptoms were mainly depressive in nature six had symptoms of anxiety neurosis.)

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EARLY MALIGNANT DISEASE OF THE CHORION

Eng Soon Teoh, S. S. Ratnam and M. Yusoff Dawood

From the Department of Obstetrics and Gynaecology (Head Prof S. S. Ratnam), University of Singapore Kandang Kerbau Hospital, Singapore 8

Abstract. Early malignant disease of the chorion recognised when month of hydatidiform mole occurred in 6% of 532 molar pregnancies. Malay patients and patients above 40 years showed an increased risk of malignancy The local and metastatic features of the disease is described. A secondary rise in serum HCG is typical. Treatment of choice is methotrexate. The overall mortality 3.9%

Considerable disagreement exists over the definition and classification of malignant growths of the chorion. (Acosta-Sison, 1959· Tow 1966 Chun & Braga, 1967 Parks, 1967). In the past a number of authors have recommended a policy of watchful waiting when evidence of residual disease is found so long as the lesion does not appear to be proliferating (Mack & Catherwood, 1930· Delfs, 1957 Smalbraak, 1957) Our Singapore experience has shown that even a short delay in instituting treatment may prove to be fatal (Teoh, 1967).

This report will present the clinical features of early malignant disease of the chorion and discuss its prognosis and management.

MATERIAL

In Singapore, the histologic demonstration of myometrial invasion, and clinical, radiographic and endocrinologic evidence of metastasis to distant sites are the accepted criteria for diagnosing malignancy of hydatidiform mole. Histologically two types of choriocarcinoma are recognised. The tumour which retains the villous pattern termed villous choriocarcinoma while the tumour which shows no evidence of villi is referred to as avillous choriocarcinoma. With increasing resort to chemotherapy as the primary treatment histological diagnosis is now rarely made in the absence of histology the lesion is termed clinical choriocarcinoma. "Early malignant disease of the chorion" refers to those cases which are discovered either at the time of diagnosis of hydatidiform mole or within month of diagnosis. The analysis is based on 51 cases which have been followed up for period of 3 to 12 years.

RESULTS

Incidence

In 51 out of 532 cases of hydatidiform mole evidence of malignancy existed when the moles were first diagnosed or malignant features became evident within a month of diagnosis. The incidence of early malignancy is thus 9.6% of all molar pregnancies. The incidence of hydatidiform mole in Singapore is 1 823 pregnancies (Teoh, Dawood & Ratnam, 1970) Hence, the incidence of early malignant trophoblastic disease is 1 8 584 pregnancies.

Age

Numerous investigators have drawn attention to the increased incidence of hydatidiform mole in teenagers and especially in elderly women (Chun et al., 1964 Tow 1966· Teoh, Dawood & Ratnam 1970). The age incidence of the 532 cases of hydatidiform mole and the 51 cases of early malignant disease of the chorion are shown in Table I. Malignant change occurred in all age groups but there is a considerable increase after the age of 40 years. In the women above the age of 40 years 14.63% of the moles developed malignancy There seems to be also slight increase in the 15–19 and 35–39 age groups when compared with the 30–34 age group.

Parity

It has been further demonstrated that the risk of developing malignancy after hydatidiform mole is

symptoms decreases (Selye, 1950) and the symptom complex becomes less dramatic.

Of course there might be underlying psychogenic causes in these patients, which explain their dieting. The possible reasons for the frequent appearance of this behaviour will be discussed in a later paper

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Considerable disagreement exists over the definition and classification of malignant growths of the chorion. (Acosta-Sison, 1959; Tow, 1966; Chun & Braga, 1967; Parks, 1967). In the past a number of authors have recommended a policy of watchful waiting when evidence of residual disease is found so long as the lesion does not appear to be proliferating (Mack & Catherwood, 1930; Delfs, 1957; Smalbraak, 1957). Our Singapore experience has shown that even a short delay in instituting treatment may prove to be fatal (Teoh, 1967).

This report will present the clinical features of early malignant disease of the chorion and discuss its prognosis and management.

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In Singapore, the histologic demonstration of myometrial invasion, and clinical, radiographic and endocrinologic evidence of metastases to distant sites are the accepted criteria for diagnosing malignancy of hydatidiform mole. Histologically two types of "chorioncarcinoma" are recognised. The tumour which retains the villous pattern is termed "villous choriocarcinoma" while the tumour which shows no evidence of villi is referred to as "avillous choriocarcinoma". With increasing resort to chemotherapy as the primary treatment histological diagnosis is now rarely made; in the absence of histology the lesion is termed "clinical choriocarcinoma". "Early malignant disease of the chorion" refers to these cases which were discovered either at the time of diagnosis of hydatidiform mole or within a month of diagnosis. The analysis is based on 51 cases which have been followed up for a period of 3 to 12 years.

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Age

Numerous investigators have drawn attention to the increased incidence of hydatidiform mole in teenagers and especially in elderly women (Chun et al., 1964; Tow, 1966; Teoh, Dawood & Ratnam, 1970). The age incidence of the 532 cases of hydatidiform mole and the 51 cases of early malignant disease of the chorion are shown in Table I. Malignant change occurred in all age groups but there is a considerable increase after the age of 40 years. In the women above the age of 40 years 14.63% of the moles developed malignancy. There seems to be also a slight increase in the 15–19 and 35–39 age groups when compared with the 30–34 age group.

Parity

It has been further demonstrated that the risk of developing malignancy after hydatidiform mole is

Table I. *Age group distribution of moles and early malignant trophoblastic disease*

Age (years)	15	15–19	20–24	25–29	30–34	35–39	> 39
No of H. moles	0	51	134	132	90	48	82
No of early malignant trophoblastic disease cases	0	6	10	13	4	6	12
% age of moles that were EMTD	0	11 75	7 46	9 85	4.4	1_5	14.63

Table II *Parity of patients with H moles and early malignant trophoblastic disease*

Parity	2 and below	3 and above
Moles	250	282
Early malignant trophoblastic disease	20 (8.0%)	31 (11.0%)

increased in patients who are para 3 or more (Chun et al 1964 Tow 1966 *a*) Early malignant trophoblastic disease was slightly more common in the patients who were para 3 and above (11%) as compared with the para 2 and below (8%) but this difference was not statistically significant (Table II)

Race

Teoh, Dawood & Ratnam (1970) have shown that there is no significant difference in the incidence of molar pregnancy among the Chinese Malay and Indian patients in Singapore The risk of developing early malignant trophoblastic disease in these racial groups is shown in Table III Malay patients had a significantly greater risk of developing early *malignant disease of the chorion* (15.8%) when compared with non Malay patients (7 1%) ($p < 0\ 02$) This was apparently not due to either increasing age or parity It is the impression of our pathologists that other tumours in Malay patients also progress more rapidly than is usual for the other races, and it is interesting to speculate that this might be related to an inadequate immunological response (K K. Tan personal communication)

Clinical features

Early malignant disease of the chorion, like late avillous choriocarcinoma, may manifest itself by its local metastatic and hormonal effects. Only a single feature may be present in a given case but in most instances the local and metastatic features are accompanied by a characteristic gonadotrophin pattern

Local effects

Myometrial invasion in an untreated hydatidiform mole is difficult to recognise unless perforation precipitates the picture of an acute abdomen. When the broad ligament is invaded, a broad ligament haematoma may be produced. After uterine evacuation there is subinvolution of the uterus and irregular bleeding persists if the growth remains exposed in the uterine cavity Such tumours may be detected by the curette but there is always a risk of provoking severe haemorrhage when the tumour is biopsied. Nevertheless, a curettage is necessary in the presence of a slow involuting bleeding uterus after hydatidiform mole because residual molar tissue may have been left behind after the first curettage

A pelvic arteriogram will show vascular patterns typical of neoplastic areas within the myometrium (Hendricks & Cockshott, 1964).

In the present series, 21 cases (41.2%) had no

Table III *Racial distribution of H moles and early malignant trophoblastic disease*

	Chinese	Indians	Others	Non Malay	Malays
No of Moles	40	35	6	443	89
No of early malignant trophoblastic disease cases	33		1	36	15

Table IV *Sites of metastasis*

Total no. of cases	51
Cases with metastasis	30 (58 8)
( ) Lungs	7
(*b*) Vagina	8
( ) Vulva	2
(*d*) Brain	1

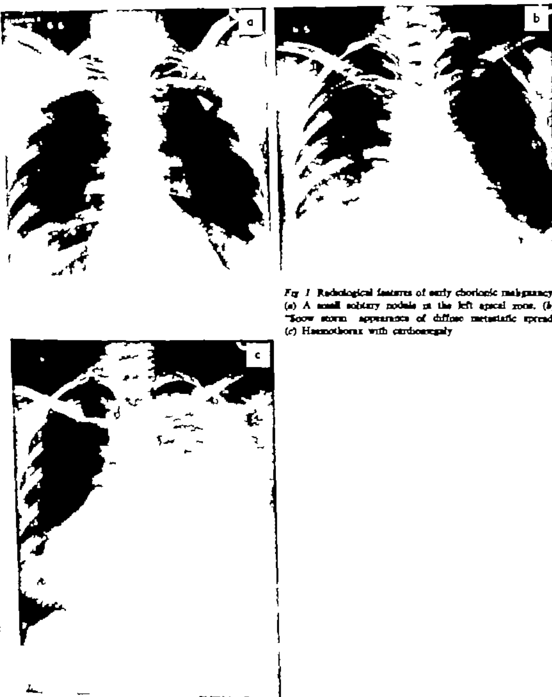


Fig. 1. Radiological features of early chorionic malignancy. (*a*) A small solitary nodule in the left apical zone. (*b*) "Snow storm" appearance of diffuse metastatic spread. (*c*) Haemothorax with cardiomegaly.

metastases. Only 6 of these 21 patients presented with persistent irregular vaginal bleeding and subinvolution of the uterus. Three cases presented with an acute abdominal episode: two with internal haemorrhage from uterine perforation and one with pelvic haematomas that resulted from parametrial invasion. All 21 cases were confirmed by curettage, laparotomy or hysterectomy.

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Local effects

Myometrial invasion in an untreated hydatidiform mole is difficult to recognise unless perforation precipitates the picture of an acute abdomen. When the broad ligament is invaded, a broad ligament haematoma may be produced. After uterine evacuation there is subinvolution of the uterus and irregular bleeding persists if the growth remains exposed in the uterine cavity Such tumours may be detected by the curette but there is always a risk of provoking severe haemorrhage when the tumour is biopsied. Nevertheless, a curettage is necessary in the presence of a slow involuting, bleeding uterus after hydatidiform mole because residual molar tissue may have been left behind after the first curettage

A pelvic arteriogram will show vascular patterns typical of neoplastic areas within the myometrium (Hendricks & Cockshott, 1964)

In the present series, 21 cases (41.2) had no

Table III *Racial distribution of H moles and early malignant trophoblastic disease*

	Chinese	Indians	Others	Non Malay	Malays
No of Moles	40	15	6	443	89
No of early malignant trophoblastic disease cases	33		1	36	15

Table IV *Sites of metastasis*

Total no of cases	51
Cases with metastasis	30 (58 8)
( ) Lungs	
(*b*) Vagina	8
( ) Vulva	
(*d*) Brain	1

were given. Parenteral methotrexate has now superseded oral chemotherapy

The results of treatment are shown in Table V The overall mortality was 3.9% In the non-metastatic group (21 patients) only 1 patient died (4.8%), from septicemia which developed during the first course of methotrexate. This death was probably avoidable and therefore the corrected mortality would be 0

In the group with metastases (30 patients) one death occurred (3.33%). The patient died from pulmonary haemorrhage 7 weeks after malignancy had been diagnosed.

DISCUSSION

It cannot be denied that the main hope for cure in cancer lies in its early detection and early treatment However in the field of trophoblastic neoplasms, the point is often made, that chorioadenoma destruens" (syn. villous choriocarcinoma") is not malignant because it has been know to undergo spontaneous resolution (Bardawil & Toy 1959 Bagshawe, 1969). Authorities with much wider experience in the Far East disagree. Acosta Sison (1959) stated that in 72% of 18 cases of chorioadenoma destruens there were complications which endangered the life of the patient She recounts with irony the lift of L.V Ackerman who was shown a slide of chorioadenoma destruens in the uterus. Ackerman voiced the opinion of pathologists that it was a benign lesion but on learning that the patient died from pulmonary and cerebral metastases a few days after hysterectomy he said, In this case the tumour is histologically benign but clinically malignant" Prawirohardjo, Martiono & Tjokrowegoro (1957) has presented a detailed protocol of 6 cases to prove that destructive mole should not be considered to be a benign tumour but is rather a malignancy which is simila to true choriocarcinoma. Their mortality rate for 16 cases of illous choriocarcinoma was 20% Tow (1966) has accepted this classification and has shown from an analysis of published reports and his ow observations that there was 35% mortality in 144 cases of chorioadenoma destruens Our own mortality rate in over 600 cases of benign hydatidiform mole is 0

The term *early malignant disease of the chorion* has been used in this study because histological data were only obtained in a third of cases, secondly in lieu of the short interval between diagnosis of hydatidiform mole and recognition of malignancy and finally to emphasize that this is an early stage in the broad spectrum of chorionic malignancy The present study has demonstrated that even with chemotherapy the mortality rate is 3.9% In the presence of metastasis, prognosis is worsened. The disease progresses rapidly to avillous choriocarcinoma if untreated, and this occurs without any warning sign or symptom (Teoh, 1967)

The recognition of early malignant disease of the chorion rests on the practice of repeated, routine, careful search by clinical examination, radiographs, and serial gonadotrophin assays. There is much to be said for routine, primary hysterectomy for hydatidiform mole in patients who are above the age of 40 years and para 3 or more inasmuch as this occasionally reveals the non-metastatic terine lesion and frequently ef fects a cure Hertz, Ross & Lipsett (1963) have shown that prognosis in choriocarcinoma is worsened with an increase in the interval between the last pregnancy and the recognition of malignancy This observation is universal and with the vailability of methotrexate and the relative safety of the drug there is no longer any justification for not treating early malignant disease with chemotherapy either alone or in combination with the appropriate surgery

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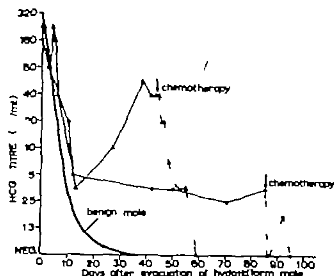


Fig Serum HCG patterns in 2 cases of early chorionic malignancy compared with the pattern in benign hydatidiform mole. Note the prolonged HCG secretion and the secondary rise in serum HCG

Metastatic effects (Table IV)

Contrary to an old belief (Hertig & Sheldon 1947) early malignant disease of the chorion disseminates freely and 30 cases (58 8%) in the present series already had metastases when they were first diagnosed. The commonest site of metastasis was in the lungs (27 cases) followed by the vagina (8) vulva (2) and brain (1) These lesions are all initially silent and pulmonary metastases must be looked for by routine chest radiographs, and vaginal metastases by repeated speculum examinations.

Three patients presented with haemoptysis, one with acute dyspnoea, and one had chest pain The typical radiographic appearance (Fig. 1 *a–c*) is small to large nodular opacities, usually present in the periphery and sometimes so small that they are difficult to distinguish from tuberculous lesions. A snow-storm appearance is also common and occasionally there may be a pleural effusion or haemothorax. Not uncommonly the heart may be enlarged with a prominent pulmonary conus and evidence of right heart failure Electrocardiographic changes typical of cor pulmonale" are evident when there is extensive pulmonary metastasis.

Secondaries in the vagina appear as plum-coloured, submucous nodules, typically in the antero-lateral aspect. When the lesion ulcerates considerable bleeding may occur

Hormonal features

The hormonal features of early malignant disease of the chorion have already been described in detail elsewhere (Teoh, 1967). Two types of gonadothrophin excretion pattern are typical, the first being a secondary rise in HCG titre and the second being a blunting off of the initial fall of HCG following the evacuation of hydatidiform mole to the level which is determined by the quantity of functional, malignant trophoblast (Fig. 2) Studies of human chorionic somatomammotrophin as an additional index of tumour activity in trophoblastic disease has thus far been disappointing (Samaan et al. 1966 Teoh, 1970).

Treatment

Treatment of early malignant disease of the chorion consists of surgery or chemotherapy Prior to 1962, methotrexate was not available in Singapore so the early cases were all treated by surgery alone. Vulval and vaginal secondaries were excised while persistent pulmonary lesions were treated by thoracotomy and wedge resection Patients above the age of 40 and those who have had enough children were considered for hysterectomy From 1962 all patients received methotrexate regardless of whether the disease was localised or metastatic Methotrexate was given in oral doses of 5 mg q i.d. until toxic symptoms appeared Courses were repeated after suitable rest periods until the immunoreactive HCG titre fell below 0.6–5 I U /ml. Then three more courses

Table V *Results of treatment of early malignant trophoblastic disease*

	Treatment	Total no.	No. in	No. deaths	Overall mortality
All cases	Surgery or Surgery & Chemotherapy	51	49	2 (3 9)	3.9
Without metastases	Surgery	1	1	Nil	4 8
	Surgery & Chemotherapy	9	8	1 (11)	
With metastases	Surgery	6	5	1 (16 7)	3.3
	Surgery & Chemotherapy	4	4	0	

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CYSTINE AMINOPEPTIDASE ACTIVITY IN PREGNANCY

I A Rapid Method for Estimation of Placental Function in Clinical Practice

Gunnar Rydén

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Abstract A rapid method for estimation of cystine aminopeptidase activity (CAP) in the serum of pregnant women is presented. The method takes only 3 hours and 10–15 samples can be estimated simultaneously. Using this method the CAP activity has been estimated in about 700 blood samples from non-pregnant and pregnant women. The CAP activity was estimated to 0.12 ± 0.012 mU/ml serum in non-pregnant women (n = 12), 0.80 ± 0.08 mU/ml serum in the 21st week of pregnancy (n = 15) and 5.72 ± 0.29 mU/ml serum in the 40th week of pregnancy (n = 30) which implies a 50-fold increase of the enzyme activity during pregnancy. The method can be a valuable parameter in judging the length of pregnancy between 21–37 week of pregnancy and preliminary studies indicate that the method might be of value to detect placental insufficiency in the third trimester.

During normal human pregnancy an enzyme appears in the blood with the ability to degrade oxytocin. This enzyme is demonstrable in neither the non-pregnant woman nor the male, nor in plasma of other species during pregnancy with the exception of the Rhesus monkey. The enzyme has been named oxytocinase. Tuppy & Nesvadba (1957) stated that the enzyme is an aminopeptidase and that l-cystine-di-β-naphthylamide (CBNA) can be used as substrate for estimation of oxytocinase activity in pregnancy.

In previous studies (Rydén, 1966) it was demonstrated that the placenta is the origin of the aminopeptidase specific for human pregnancy. It was also found that CBNA is degraded by other enzymes in blood and this may influence the estimation of the enzyme activity specific for human pregnancy.

Sjöholm (1964) and recently Yman (1970) have purified oxytocinase from retroplacental blood and tested this purified oxytocinase against different substrates. They concluded that oxytocinase is an aminopeptidase with broad specificity. From the above-mentioned reasons it seems more accurate to name the enzyme activity in blood according to the substrate used. With l-cystine-di-β-naphthylamide as substrate the activity in serum should therefore preferably be named cystine aminopeptidase (CAP) instead of oxytocinase activity.

The purpose of the present investigation is to present a simple method for estimation of CAP activity in serum and to test its value in clinical practice as a measure of the functional state of the placenta.

MATERIAL AND METHODS

Blood samples from pregnant and non-pregnant women were centrifuged at 900 g for 10 min, the supernatant withdrawn and then stored in a refrigerator at +4°C if used within 24 hours. Otherwise the samples were kept at −20°C until use. In preliminary experiments it was demonstrated that storing the samples at +4°C for 72 hours causes a 10% loss of enzyme activity.

Substrate solution. 150 mg cystine-di-β-naphthylamide (Mann Research Labs., New York) was dissolved in 60 ml 0.012 N HCl and diluted with water to 100 ml during moderate warming and stirring.

Buffer solution. Phosphate buffer 0.067 M, pH 7.4, was used.

Colour reagent. Fast Garnet Reagent (Chroma Gesellschaft, Stuttgart, BRD) 0.5 mg/ml was prepared daily. 50 mg Fast Garnet Reagent was dissolved in 100 ml 1.0 M acetate buffer, pH 4.2, containing 10% (w/v) Tween 20.

Other reagents. Trichloroacetic acid (TCA) 10% (w/v). Standard β-naphthylamine solutions (10 μg/ml) were diluted from a stock solution prepared from 100 mg of

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Substrate solution. 150 mg cystine-di-β-naphthylamide (Mann Research Labs, New York) was dissolved in 60 ml 0 012 N HCl and diluted with water to 100 ml during moderate warming and stirring.

Buffer solution Phosphate buffer 0 067 M, pH 7 4 was used.

Colour reagent. Fast Garnet Reagent (Chroma Gesellschaft, Stuttgart, BRD) 0.5 mg ml was prepared daily 50 mg Fast Garnet Reagent was dissolved in 100 ml 1 0 M acetate buffer, pH 4.2, containing 10% (w/v) Tween 20

Other reagents Trichloroacetic acid (TCA) 10% (w/v). Standard β-naphthylamine solutions (10 μg ml) were diluted from stock solution prepared from 100 mg of

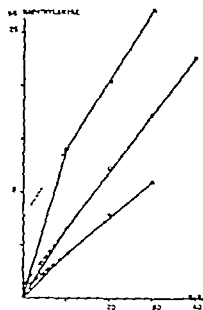


Fig 1 Progress curves for the hydrolysis of cystine-di-β naphthylamide in three different serum samples from pregnant women.

naphthylamine dissolved in 1 000 ml 0.01 N HCl. As far as possible, all reagents were of analytical grade

Incubation mixture The serum samples were diluted (1+3) with phosphate buffer pH 7.4 1 ml of diluted serum was mixed with 0.5 ml substrate solution in three different test tubes. One served as zero control and 0.75 ml TCA was added immediately into it. The remaining tubes were incubated in a water bath at 37°C for different lengths of time, usually 60 and 120 min during constant shaking. This procedure is necessary as the substrate is soluble only to a very small extent in the incubation mixture Some preliminary experiments were performed where Tween 20 in different concentration was added to the incubation mixture This increased the solubility of the substrate but inferred with the enzyme kinetics and could therefore not be used.

The degradation of cystine-di-β-naphthylamide (CBNA) was interrupted by adding 0.75 ml TCA to the samples. The tubes were centrifuged at 900 g during 6 minutes. 1 ml of the supernatant was added to ml Fast Garnet solution and incubated 15 min at 37°C The samples were read immediately in a spectrophotometer at 510 nm. The colour reaction is unstable and storing of the samples for more than 30 min causes a lowering of the extinction values. The amount of naphthylamine in the samples was calculated from standard curve. The colour reaction follows Lambert-Beer's law for naphthylamine concentrations in the range of 0.3 to 3 μg ml.

Calculation of the CAP activity According to the recommendations in the Report of the Commission on Enzymes of the International Union of Biochemistry (1961) one enzyme unit is defined as that amount of enzyme which will catalyze the transformation of one μM substrate per minute under defined conditions. If more than one bond is acted upon, which is applicable to CBNA, 1 μequivalent of the actual substrate should be used instead of μM This calculation is the same as used by Klimek & Malolepszy (1969). Melander (1965) and Rydén (1966) defined the enzyme unit as that amount which catalysed the transformation of one M CBNA per minute. Their figures should therefore be multiplied by the factor 20 in order to be comparable with the present ones. The standard deviation of the CAP assays was calculated from the duplicate estimations performed. The coefficient of variation was ±1 to ± the higher value was found for the higher extinction values.

RESULTS

In a series of experiments serum samples were incubated with CBNA for different lengths of time. The results are shown in Fig. 1 A marked deviation from the linear in the progress curve was found during the first 30 to 60 min in many samples, but after that time the reaction was of zero order This deviation from the linear was also found if the samples were preincubated at 37 C for 60 min before addition of the substrate. In order to determine the enzyme activity under standardized conditions the serum samples have been incubated 60 and 120 min with CBNA. The CAP activity has been calculated from the

Table I *CAP activity in serum of pregnant and non-pregnant women*

		Enzyme activity in mU ml serum	
		Mean	S D
Non-pregnant	1	0.1	0 04
Week of pregnancy			
1	15	0 80	0 30
2	23	1 00	0.34
3	20	1 10	0 4
4	19	1 8	0 36
5	11	1 28	0 46
6	1	1 14	0 28
7		1 64	0 56
8	16	1 76	0 68
9	11	34	0 84
30	3	68	1 76
31	1	34	0 78
3	4	56	0 66
33	37	74	1 36
34	30	3 40	1 3
35	41	4 02	1 56
36	77	4 10	1 90
37	57	4 3	1 36
38	39	5 76	1 54
39	30	5 84	1 70
40	36	5 77	1 74
41	36	5 60	1 60
4	3	5 76	1.86



Fig. 2. CAP activity in normal pregnancy. The curve represents the arithmetic mean values and the standard deviation

slope of the progress curve between 60 and 120 min. Using this method the CAP activity has been estimated in about 700 blood samples from healthy non-pregnant and pregnant women. The results are listed in Table I and Fig. 2, where the arithmetic mean values and standard deviation is given. The median values and the figures between the 10th and 90th percentile have also been calculated and the results are shown in Fig. 3.

In 75 cases the CAP activity was estimated in blood samples taken during labour from patients between 39 and 41 weeks of pregnancy. The CAP activity was correlated to the weight of the placenta and the birth weight. A positive correlation was found between placental weight and CAP activity. The results are shown in Fig. 4. No such correlation could be demonstrated between CAP activity and birth weight.

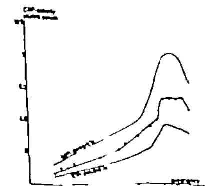


Fig. 3. CAP activity in normal pregnancy. The curves represent the median values and the values between the 10th and the 90th percentile

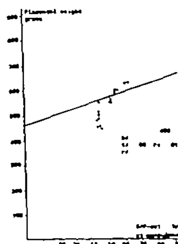


Fig. 4. The correlation between CAP activity in serum at delivery and placental weight. CAP activity is expressed in µg naphthylamine/ml serum and hour

DISCUSSION AND CONCLUSIONS

As the placenta is the main source of CAP activity in the blood of pregnant women, it has been suggested that estimation of CAP activity should reflect the functional state of the placenta (Rydén, 1966; Babuna & Yenen, 1966). Clinical reports also show that the activity is increased in twin pregnancy (Melander, 1965; Ichaliotis & Lambrinopoulos, 1965). Investigations concerning CAP activity in toxaemia of pregnancy and intrauterine foetal death are however conflicting. Some authors (Ichaliotis & Lambrinopoulos, 1964; Babuna & Yenen, 1966) have found decreased values in these conditions, whereas Melander (1965) was unable to confirm these results. The different results reported using Tuppy & Nesvadba method (1957) may be explained by the fact that this method is troublesome and is difficult to carry out accurately. Thus the substrate is very poorly soluble which, under certain conditions, influences the kinetics of the reaction, (Rydén, 1966). The coupling of the released naphthylamine into an azodye is very time-consuming and recently Klimek & Malolepszy (1969) have shown that this reaction is unstable. This has not always been taken into consideration and must of course influence the results.

In the present study a marked deviation from the linearity in the progress curve was found during the first 30–60 min in many samples, but after that time the reaction was of zero order

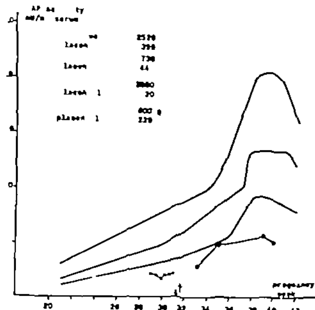


Fig 5 CAP activity in 3 cases where the babies were born "small for date and in one case of intra-uterine fetal death. The arrow indicates the time of delivery f the stillborn baby The fetal heart sounds disappeared 36 hours before delivery

This has also been found recently by Tovey *(1969) using 1-cystine-bis p-nitroanilide as substrate* In previous experiments Rydén (1966) demonstrated that non pregnant as well as pregnant sera could degrade CBNA enzymatically In non-pregnant sera, however the progress curve was not linear and the explanation was that the enzyme responsible for the degradation of CBNA in non pregnant sera was inactivated gradually during the incubation.

However preincubation of the samples for 60 min before addition of the substrate did not change the progress curve This means that the substrate per se must influence the enzyme activity *Purified cystine aminopeptidase (oxytocinase)* is stable at 37 C and the reaction using the substrate CBNA is of zero order (Yman & Sjöholm 1967) It seems probable, therefore that the deviation from the linear is due to other aminopeptidases or amidases in serum Consequently it must be more accurate to calculate the CAP activity from the slope of the progress curve between *60 and 120 min, as after that time the* main source of CAP activity in serum is the placenta.

Using this method for estimation of CAP activity in pregnancy the mean value in the last *month of* pregnancy is very similar to that found by Müller Hartburg, Nesvadba & Tuppy (1959) In fact they calculated the enzyme activity to be 5 60 mU/ml serum in the last month of pregnancy which is almost exactly the same as found in the present study in the 40 week of pregnancy 5 72 ± 0.29 mU

The CAP activity *increased 50 times during* pregnancy as compared with the non-pregnant state whereas in the studies by Müller Hartburg, Nesvadba & Tuppy (1959) and Melander (1965) the increase was 30 and 20 times respectively

In certain pathological conditions aminopeptidases from other sources may influence CAP level and give false high values in serum. This has been demonstrated by Müller Hartburg, Nesvadba & Tuppy (1957) in sera from patients with liver diseases. Rydén (1966) has shown that the CAP activity of rat liver extracts has the same characteristics as that found in non-pregnant human sera. In certain pathological conditions i.e. liver diseases, severe toxaemia of pregnancy false high values of CAP might be found in the blood. This implies that the value of CAP assays in such conditions is questionable

At present, estimation of *urinary oestriol* is most generally used for monitoring the condition of the placenta and the fetus. Most of the published methods for urinary oestriol assay are, however either laborious and time-consuming *or of insufficient specificity* Recently a new rapid method for oestriol assays has been published by de la Torre Johannisson & Diczfalusy (1970) The method takes only *5 hours but* still requires the collection of a urine sample. They suggest however that a full 24 hour specimen of urine may not be required provided the oestriol content of the urine is related to the *concentration of endogenous creatinin.* From clinical practice, it is a well-known fact that oestriol assay may vary considerably from day to day in the same patient. This is a drawback of the oestriol method and for that reason it should be of value to have another method of evaluating placental function

Babuna & Yenen (1966) have suggested that *estimation of CAP activity in blood* can be used as a test of placental function Tovey (1969) and Kleiner Brouet Yager & Graff (1969) using other substrates for estimation of aminopeptidase activity in pregnancy ha e reached the same conclusion In the present investigation a steadily increasing CAP activity was found during preg-

nacy and there was a correlation between CAP activity at term and placental weight. This may indicate that CAP can be used to measure placental function.

On reviewing the results it was noted that some very low CAP values were found in cases of the small for dates syndrome. These cases are described in Fig. 5

The diagnosis was established by the paediatricians without knowledge of the CAP values. The results are only preliminary and further studies are in progress to evaluate the clinical significance of these findings.

From a practical point of view estimation of CAP in blood is much easier to perform than oestriol assays in the urine. Whereas oestriol estimation reflects the feto-placental unit as a whole CAP is primarily a measure of the functional capacity of the placenta, and changes in oestriol excretion and CAP activity may not occur simultaneously. No such comparison has been performed and must be done before the value of CAP in clinical practice can be established.

Another important point is how fast a decrease in placental function is reflected by a decrease in CAP activity in blood. Babuna & Yenen (1966) demonstrated a 50% decrease in CAP activity 1 day after intra-uterine fetal death, whereas in the studies by Melander (1965) and Tovey (1969) the decrease was more gradual. The enzyme responsible, oxytocinase, is an aminopeptidase with high molecular weight, about 300 000 according to Yman (1970) and is not excreted in the urine. Changes in placental function might therefore be reproduced rather slowly in the blood.

Tryding & Willert (1968) have suggested that determination of plasma diamino oxidase (histaminase) by a radiochemical micromethod can be of value for estimating the length of pregnancy during the first half of pregnancy. The diamine oxidase activity increases very rapidly during the first 20 weeks of pregnancy but thereafter is nearly constant. Owing to the steadily increasing CAP activity between the 1st and 37th weeks of pregnancy CAP assay can be of value for determining the length of gestation in uncertain cases.

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A ROUTINE CLINICAL METHOD FOR THE ESTIMATION OF LOW POLAR OESTROGENS IN HUMAN URINE

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Abstract A simple, rapid and sufficiently specific method for the estimation of low polar oestrogens in human non-pregnancy urine is described. It involves hot acid hydrolysis, ether extraction, simple, highly effective alkaline washing, isolation of the phenolic fraction, saponification, methylation and treatment with hydrogen peroxide. The methylated low polar oestrogen fraction is further purified by washing procedure and finally subjected to adsorption chromatography on aluminium oxide. Quantitation is made by Kober colorimetry During five day orking week one technician can maintain daily output of more than ten analyses. The method is well suited for general clinical routine use By omission of the adsorption chromatography the method is converted into rapid procedure in which the analytical answer can be given in less than one orking day. This variant can be used when rapid answers are necessary and when slight "overestimation" is not important, e.g. monitoring gonadotrophin stimulation therapy

The introduction of Brown's (1955 1957) chemical method for the estimation of oestrogens in non-pregnancy urine initiated the development of several methods for this purpose. Many of them are based upon the original Brown procedure, thereby demonstrating the soundness of the basic method. These methods are often methodologically "stable and in some cases (Brown & Blair 1960; Exley & Corker 1965; Frandsen, 1967) highly specific. They re mostly time consuming and laborious, including the handling of large amounts of reagents.

Methods for the estimation of total urinary oestrogens, based upon the Ittrich (1958) extraction procedure are ery rapid and convenient, but some of them, including the original Ittrich procedure, lea e ery much to be desired with regard to their specificity. An improved dev lopment of the Ittrich procedure is the total oestrogen method of Brown et al. (1968). This method has been used successfully for monitoring ovulation induction with gonadotrophins (Brown et al., 1969; Gemzell et al., 1970). It has some disadvantages, however which might affect its methodological stability. The Ittrich extraction procedure is dependent upon critical temperatures (below 0 C) and the colour complex is rather shortlived. Also a highly sensitive (and highly expensive) fluorimeter is necessary. This fact makes an extensive supervision necessary especially when less trained technicians are used.

I this laboratory a slight modification of the original Brown method was used until 1967 (Furuhjelm & Waller 1958). As has been reported for the Brown method (Brown & Blair 1960; Frandsen, 1967), this modification in some cases also gave peculiar results, over-estimation as well as negative alues. This occurred even when the saponification procedure of Bauld (1956) was included in the method.

I 1967 the method was modified to tak only the oestrone + oestradiol" fraction. With this limitation a simplified ether extraction and a more efficient alkaline purification could be used. This resulted in the following modified method: 200 ml urine is hydrolysed according to Brown (1955). Extract with 120 ml ether. Wash the ether phase with 8% sodium bicarbonate (discard), concentrated carbonate buffer pH 10.4 (discard), 2 M sodium hydroxide (discard, *no* addition of sodium bicarbonate and re-extraction), 8% sodium bicarbonate (discard) and water (discard). Evaporate the ether phase. After that the sample is processed xactly as the oestrone + oestradiol fraction in the modified method of Brown (1957) and the oest

capacity of 150 ml. The "small" tubes are of the same shape but with a diameter of 30 mm and a capacity of 60 ml. The small tubes are handled in rotating shaking racks with a capacity of 20 or 40 tubes. All the phases to be discarded (lower phases) are removed by suction. This equipment, originally developed by Frandsen (1963) is suitable for several rapid extraction procedures.

The chromatography columns are glass tubes of 10 mm internal diameter fitted with a capillary at the lower end and a wider reservoir with an internal diameter of 20 mm at the upper end. The aluminium oxide is distributed with a small calibrated measuring cylinder.

Standardization of the aluminium oxide. The aluminium oxide is standardized according to the principles of Brown (1955) in such a way that the oestrone- and oestradiol-3-methyl ethers will elute quantitatively with the benzene. Using the oxide manufactured by E. Merck, Darmstadt, Germany (Aluminiumoxid, standardisiert zur Chromatographischen Adsorptionsanalyse nach Brockmann, Aktivitätsstufe II III) we usually add 3% of water.

Chemicals. With exception of the diethyl ether all reagents are of A.R. grade. The organic solvents are distilled, benzene and n-hexane over KOH pellets. Before use benzene and n-hexane are saturated with water. Dimethyl sulphate must be waterclear and colourless, in other cases it has to be purified by means of simple distillation.

The diethyl ether used in this procedure is of technical grade ("Laboratory ether" A.B. Bofors, Bofors, Sweden) and is distilled over 1/10 vol of a 50% (w/v) solution of ferrous sulphate in 5% sulphuric acid and used the same day. Contrary to what has been reported by other authors (Ittrich, 1962; Frandsen, 1967) we have no bad experiences with cheap quality ("technical" or "laboratory") diethyl ether.

Concentrated carbonate buffer is prepared according to Brown (1955): 150 ml of 5 M sodium hydroxide plus 1000 ml of 8% (w/v) sodium bicarbonate.

Sulphuric acid concentrations: 40%, means 400 ml of concentrated sulphuric acid plus 600 ml water.

COMMENTS ON THE METHOD

Hydrolysis. The hydrolysis is carried out according to Brown (1955). The principle of Frandsen (1967) involving autoclaving with sulphuric acid will give practically identical results with this method and may be used as well.

Extraction and purification. The small volume of ether used is sufficient for extraction of the low polar oestrogens being determined. The effect of the improved alkaline washing will be dealt with under "Results". It is an absolute prerequisite for the specificity of the method.

Isolation of the phenolic fraction. The partition system used in this method is taken directly from the method of Frandsen (1967). The technical advantage of using chlorinated hydrocarbons with high specific gravity instead of mixtures of benzene and hexane has also been pointed out by that author. The saponification step is taken from the method of Hirvonen et al. (1968). It is more rapid but less effective than the original procedure of Bauld (1956). In spite of that we have observed a significant degree of purification of certain urines by this simplified variant.

Methylation and oxidation. This step is carried out according to the principles of Brown (1955). The washing of the hexane extract with 40% methanol and 40% sulphuric acid is taken from the method of Frandsen (1967) who also pointed out the considerable degree of purification offered by this step.

Adsorption chromatography and Kober colorimetry. The adsorption chromatography closely follows the principles of Brown (1955). As standard for the colorimetry a mixture of oestrone 3-methyl ether and oestradiol-3-methyl ether 2:1 is used. The reason for this will be dealt with in the discussion. Sulphuric acid from different manufactures will give somewhat different specific colour intensities. The best results (i.e. highest colour intensity) were obtained with the sulphuric acid from E. Merck A. G. Darmstadt, Germany (Schwefelsäure zur Analyse, sp. Gew. 1.84). With extracts from 50 ml urine adliquots, the use of long path (4–5 cm) optical cells will be necessary at least when a common routine spectrophotometer (*Beckman B* or equal) is used.

GAS CHROMATOGRAPHIC AND MASS SPECTROMETRIC TECHNIQUES FOR THE SPECIFICITY STUDY

The gas chromatograms illustrated in Figs. 3–5 were obtained on a Perkin-Elmer F 11 gas chromatograph with flame ionization detector. The column used was a 2 m × 3 mm i.d. glass, packed with 2.5% OV 17 on AW DMCS Chromosorb W 80–100 mesh. Column temperature 235 C, nitrogen carrier gas inlet pressure 3 kg/cm².

The gas-chromatography-mass spectrometry was performed on a λ-17 gas chromatograph-mass spectrometer built at the Mass Spectrometric Laboratory, Karolinska Institutet, Stockholm, Sweden. The column used was a 3 m × 3 mm i.d. glass, packed with 1% OV 17. Column temperature 250 C, helium carrier gas flow 25 ml/min. This equipment gave chromatograms practically

rone and oestradiol are estimated together by colorimetry

After this change we had practically no trouble with the oestrogen estimations. The reason for that lies in the improved alkaline washing of the ether extract which was the crucial point of this modification as well as of the method described in this paper However including several extractions in individual separatory funnels it was rather time-consuming. In the last year it has therefore been extensively modified. The final method which is now in routine use at this laboratory is a scaled down version of the method from 1967 mentioned above, in which some valuable parts from the methods of Frandsen (1967) and Hirvonen et al. (1968) have been included. The possibility of changing the method into a rapid procedure in which the analysis can be completed in one working day has also been investigated.

In the text the designation LPE (Low Polar Oestrogens) will be used for the "oestrone + oestradiol" fraction The reason for this will be dealt with in the discussion.

List of abbreviations

GLC – Gas liquid chromatography
GLC–MS – Gas liquid chromatography – Mass spectrometry
LH – Luteinizing hormone.
LPE – Low Polar Oestrogens (in human urine mainly oestrone and oestradiol 17β).
3 MOE$_1$ – Oestrone 3-methyl ether
3 MOE$_2$ – Oestradiol-17β 3-methyl ether
3 MOE$_2$-17βOAc – Oestradiol-17β 3-methyl ether 17β acetate
TLC – Thin layer chromatography

THE METHOD IN DETAIL

Hydrolysis and extraction. 50 ml of 4 h urine is refluxed with 8 ml of conc. hydrochloric acid for 60 min Cool in tap water The hydrolyzed urine is extracted with 30 ml diethyl ether in a large extraction tube (see technical details). The urine layer is removed by suction and the ether phase is transferred to small extraction tube (see technical details). The ether phase is washed with the following solutions (every portion is discarded by suction before the next is added): 5 ml 8% (w) sodium bicarbonate 5 ml conc. carbonate buffer pH 10.4 1.5 ml M sodium hydroxide, .5 ml 8% sodium bicarbonate and .5 ml f deionized water The ether is finally evaporated at 40°C.

Isolation of the phenolic fraction. The dry extract is dissolved in 70 ml 1 M sodium hydroxide (rm at 50°C for 5 min with occasional shaking). After cooli g to room temperature the solution is ashed th 10 ml of carbon tetrachloride The sodium hydroxide phase is transferred to a clean small extraction tube and heated in a boiling water bath for 30 min. Cool in tap w ter

Methylation and oxidation. 0.9 g of boric acid is added to the solution, which is warmed at 37°C until the boric acid is completely dissolved. After that 0.4 ml dimethyl sulphate is added, the tube is shaken and left at 37°C for 15 min. ml 5 M sodium hydroxide and second portion of 0.4 ml dimethyl sulphate is added, the tube is shaken and left at 37°C for 15 min. Cool in tap water

To the methylation mixture 5 ml 5 M sodium hydroxide and 1 ml 30 hydrogen peroxide are added, the tube is shaken and left for 1 min. The oestrogen-3-methyl ethers are now extracted with 10 ml *n*-hexane The alkali layer is removed by suction and the hexane volume is brought up to 25 ml. The hexane phase is ashed with 5 ml 40% methanol, with 5 ml 40% sulphuric acid and t ice with 5 ml of water

Adsorption chromatography The hexane solution is transferred to column of g standardized aluminum oxide, packed in *n*-hexane (see technical details) The oestrogen 3-methyl ether fraction is eluted ith 25 ml benzene which is collected in a Kober tube.

For a more rapid procedure this adsorption chromatographic step is omitted. To the washed hexane extract 4 mg hydroquinone in 0.. ml ethanol is added and the extract is aporated to absolute dryness under reduced nitrogen pressure After that Kober colorimetry is carried out as described below

Kober colorimetry To the benzene eluate 4 mg of hydroquinone in 0.. ml ethanol is added and the eluate is ev porated to absolute dryness under reduced nitrogen pressure at 70°C. After cooling to room temperature 4 ml 66% sulphuric acid with % (w) hydroquinone added, and after thorough mixing the tube is placed in a boiling water bath for 20 min (shake thoroughly after and 7 min) The tube is cooled in tap ater 14 ml of ater is added and the tube is heated again in the boiling w ter b th for 10 min. After cooling in the tap w ter for 10 min the sample is read in the spectrophotometer T o standards containing m ture of 0.6 µg oestrone 3-methyl-ether and 0.3 µg oestradiol-3-methyl ther and tw Kober blank are treated in the same w

For the spectrophotometry Beckman B instrument with 5 cm light path (Agner) cells is used. Th colours are read gainst blank at 480-516-5 nm and optical densities re corrected according following equation

E $_cE_s$ $(E_{48}$ $E_{555})$

The lues of oestrogen excretion are calculated accord g t the formula

$$\mu g \ 4\,h \quad \frac{E^{sample}}{E^{standard}} \quad \frac{\mu g_{standard}}{50} \quad V$$

here µg standard is he sum of oestrone and oestradiol-3-methyl ther in the standa d (0.9) nd V the 4 hour volume in ml.

Technical details

Glassware The "large extraction tubes re up-bottomed glass stoppered tubes ith diameter of 36 mm and a

Fig. 2. Kober spectra of oestrogen standard mixture and two urine pool extracts obtained by the complete method.

Fig. 4. Gas chromatogram of urine pool extract (acetylated) after TLC. LPE fraction.

Specificity (complete method including adsorption chromatography)

Direct indications. Kober spectra from final urinary extracts and from oestrogen standards are similar in shape and have an absorption maximum at 516 nm (Fig. 2). The high titre urine from Fig. (ovulatory peak sample pool, 58 µg LPE/l) was used in a gas chromatographic-mass spectrometric specificity study. Two 200 ml aliquots were processed according to the complete method, but with the ether extraction and washing part scaled up four times. After adsorption chromatography the extracts were acetylated with acetic anhydride/pyridine 5:1. One extract was subjected to GLC and GLC-MS without any further purification (Fig. 3). The other extract was chromatographed on a Silica gel GF thin layer plate (200 × 100 mm) with ethyl acetate-benzene-n-hexane 5:4:4 as solvent. After TLC

Fig. 3. Gas chromatogram of urine pool extract (acetylated) before TLC. Details given in the text.

Fig. 5. Gas chromatogram of urine pool extract (acetylated) after TLC. "Non polar" fraction.

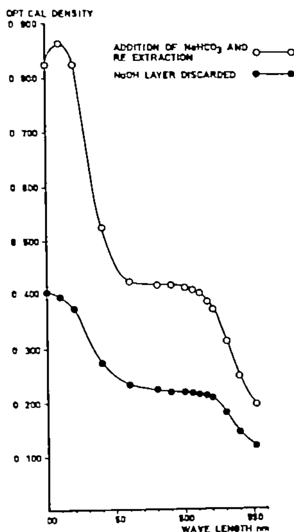


Fig 1 Kober spectra of urine pool extract obtained with the complete method with and without improved alkaline washing.

identical to those obtained on the 2.5% OV 17 column The energy at the bombarding electrons was 22.5 eV Scans were made in *m/e* range 0–500.

RESULTS

Alkaline washing of ether extract

Discarding 2 M sodium hydroxide used in the "clean up of the ether extract, without lowering the pH and re-extraction, will offer a remarkable degree of purification in a very simple way This is clearly illustrated in Fig. 1 Taking the optical density at 400 nm as a measurement of impurities, the improved alkaline washing procedure will reduce the final amount of these substances with about 50% in the present method. These impurities represent an overestimation of 7 7 µg/ 2 h in the example given in Fig. 1 In our method of 1967 this effect was even more striking, the re-extraction gave an overestimation at 13.8 µg/ 24 h in the same specimen. This might be explained by the absence of sulphuric acid washing in the earlier method. The over all recovery of the present method is not affected to any notable degree by this improved alkaline purification. This partition step has been used by Behrg (1964) for the separation of the oestrone + oestradiol and oestriol fractions.

Table II. *Precision of the method*

LPE concentration µg/24 hr	N	Estimate of S.D µg/24 hr
0 6–2.4	6	±0.43
2.8–4 7	11	±0.45
5.0–9.3	31	±0.48
10.0–76.0	37	±0.56

Recovery precision and sensitivity (complete method including adsorption chromatography)

The over all recovery of standards added to the urine immediately after hydrolysis is given in Table I The precision of the method is obtained from duplicate determinations and is given in Table II From the figures given in Tables I and II this method can be assumed to estimate LPE excretion values down to 2–3 µg 24 h with a reasonable degree of precision.

Table I *Recovery of standards added to urine immediately after hydrolysis. Quadruplicate estimations*

Urine	Added standard	Recovery	
Pool 11 7 µg litre	Oestrone corr to 1 6 µg litre	76.–	(66.8–90 0)
Pool 11 7 µg litre	Oestradiol corr t 20 6 µg litre	79 3	(69 0–89 0)
Pool 3 9 µg litre	Oestrone corr to 2.14 µg litre	81 7	(62 0–176 0)
Pool 3 9 µg litre	Oestradiol corr t 2.06 µg litre	83.2	(63.0–1.3 0)

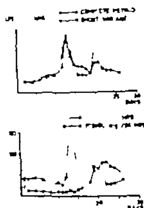


Fig. 8 Excretion of LPE measured with the short method and the complete method and of urinary LH and pregnanediol during normal menstrual cycle.

urinary extracts obtained by the complete method contain oestrone and oestradiol-17β as their 3-methyl ethers

Comparison of the complete method with its predecessor from 1967 and with the short modification without chromatography

LPE excretion values obtained by the complete method proposed in this paper closely compare with corresponding values obtained by its more complicated predecessor from 1967 (Fig. 9). The modification without chromatography mostly gives somewhat higher values than the complete method (Fig. 8 and 10).

Practicability

During a five day working week one technician can perform at least ten analyses daily with the complete method or with the short variant. With the short variant the analyses can be completed in one day. While both methods are identical up to the adsorption chromatography they can be processed on the same routine line without changes in equipment or reagents.

DISCUSSION

In routine clinical work, estimation of oestrone or oestrone + oestradiol in most cases will offer as much of clinical information as an estimation of all three "classical" oestrogens (Adlercreutz et al. 1967, Frandsen, 1967, Ilmonen et al. 1968). Such a limitation will give a shorter and more simple procedure and will allow a more efficient purification of the extract.

The complete method proposed in this paper will, down to its sensitivity level, have a higher degree of specificity and will be more rapid and

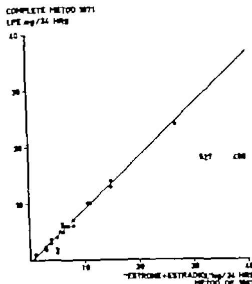


Fig. 9 Comparison of LPE values obtained by the complete method proposed in this paper and with its more complicated predecessor from 1967

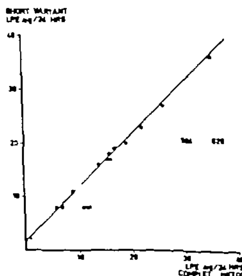


Fig. 10 Comparison of LPE values obtained by the complete method and with the short modification without adsorption chromatography

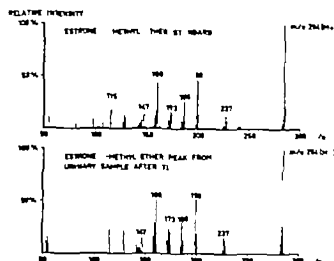


Fig 6. Mass spectrum of oestrone-3-methyl ether standard and of oestrone-3-methyl ether peak in fig. 4

the plate was divided in one zone containing 3 MOE_1 and 3 MOE_2–17βOAc (R_F 0.53–0.70) one zone with R_F 0.71–1.00 and one zone with R_F 0–0.52. They were eluated separately and analyzed by GLC and GLC MS (Figs. 4 and 5)

In the crude acetylated extract (Fig. 3) two distinct peaks with retention times corresponding to 3 MOE_1 and 3 MOE_2–17βOAc appeared. GLC MS analysis of peak A resulted in mass spectra similar to that of 3 MOE_1 but with large interfering peaks. Mass spectra recorded on peak B corresponded to that of 3 MOE_2–17βOAc. After TLC the ratio between the 3 MOE_1 peak and the 3 MOE_2–17βOAc peak was greatly reduced (Fig. 4). Mass spectra recorded at three points of the peaks were similar to those of standards and showed no major interfering peaks (Figs. 6 and 7)

By the TLC a compound with the same GLC retention time as 3 MOE_1 was separated in the zone with R_F-values 0.71–1.00 (Fig. 5). Mass spectrum of this compound and of that corresponding to peak C in chromatogram I were very different from those of the oestrogens. These two compounds were not further studied

Indirect indications. Indirect evidence for the specificity can be obtained by comparing the method with other methods and by examining its behaviour and results in routine clinical work.

Compared with the Brown (1955 1957) procedure this method contains two additional purification steps: Improved alkaline washing and washing of the hexane extract with 40 % methanol and 40 % sulphuric acid. This will result in increased purity of the final extracts.

With the method proposed in this paper as well as its predecessor from 1967 high over-estimations and negative values after Allen correction are extremely rare. Such pitfalls were rather common with the original Brown procedure as well as the modification by Furuhjelm & Waller (1958)

Good indirect evidence for the specificity of the method is obtained from the results of estimations performed on normal cycles together with estimations of LH and pregnanediol. The urinary LH excretion was estimated by the radioimmunosorbent technique of Wide (1966 1969) and pregnanediol by the method of Carlström & Furuhjelm (1970) In such cycles the LPE excretion follows the typical biphasic pattern with the first LPE peak coinciding with the LH peak (Fig. 8).

From these results it can be concluded that

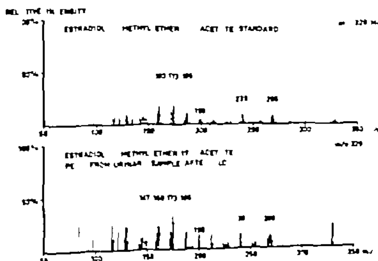


Fig 7 Mass spectrum of oestradiol-3-methyl ether 17β-acetate standard and of oestradiol-3-methyl ether 17β-acetate peak in fig. 4

22. Wide, L.: Radioimmunoassays employing immunosorbents. Karolinska Symposia on Research in Reproductive Endocrinology 1st Symposium Immunoassay of gonadotrophins. Stockholm, 1969 (ed. E. Diczfalusy).

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simple than the original Brown (1955, 1957) procedure. Also compared with methods previously developed for the estimation of oestrone or oestrone + oestradiol (Brown & Blair 1960; Exley & Corker 1965; Frandsen 1967; Hirvonen et al. 1968) it will offer a simplified procedure. The first three methods are highly specific but much more laborious than our method. The method of Hirvonen et al. (1968) is somewhat more cumbersome than our method and gives somewhat less pure final extracts. The complete method proposed in this paper is well suited for general routine clinical use.

The short variant without adsorption chromatography will give values in the same range as the complete method, with a slight "over-estimation" (Fig. 10). It can be used when the analytical answer has to be given in one day and when such an over-estimation has no clinical importance. It is more laborious and time-consuming than the total oestrogen method of Brown et al. (1968) but it is less dependent on personal and technical factors and does not require a high-cost fluorimeter. Its main advantages is that it can be processed on exactly the same routine line as the more specific complete method.

The oestrogen fraction measured by our complete method roughly corresponds to the combined oestrone and oestradiol fractions from the classical Brown (1955, 1957) method. It is well known that human urine contains other natural oestrogens in the same polarity range accompanying oestrone and oestradiol 17β (Adlercreutz & Luukkainen, 1969; Adlercreutz, 1970). It must also be kept in mind that the rather ungentle treatment of the urine (hot acid hydrolysis, heating with alkali etc.) might result in structural alternations of other oestrogens. This will include dehydratation of hydroxylated oestrogens (oestriols) giving less polar derivatives with an additional double bond, and also some epimerizations might take place. It is likely that some of these substances will accompany the oestrone and oestradiol 17β even after adsorption chromatography. We therefore find the designation "Low Polar Oestrogens (LPE)" more adequate than "oestrone + oestradiol 17β". However oestrone and oestradiol-17β will be the major steroid oestrogens in this fraction. The ratio between urinary oestrone and oestradiol 17β given by different workers will vary considerably but its mean value is approximately 2 : 1 (cf. Diczfalusy & Lauritzen, 1961). We therefore apply this ratio to the standard mixture used for the colorimetry.

From the clinical point of view the interference of low polar oestrogens other than oestrone and oestradiol-17β seems to be of very limited importance.

ACKNOWLEDGEMENTS

An antiserum coated Sephadex preparation for the radioimmunosorbent assay of LH was kindly supplied by Dr Leif Wide, University Hospital, Uppsala, Sweden. The gas chromatography-mass spectrometry was performed at the Mass Spectroscopic Laboratory (Dr Ragnar Ryhage), Karolinska Institutet, Stockholm, Sweden. Skilful technical assistance was provided by Mrs Berit Ståhl.

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A RADIOTELEMETRIC STUDY OF UTERINE ACTIVITY IN LABOUR

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Abstract. The radiotelemetric investigation of intra-uterine pressure was carried out in 904 women and proved to be a method of studying uterine activity which is harmless for both mother and fetus and can be carried out at all stages of labour. The investigation of intra-uterine pressure allows uterine contractions to be judged both qualitatively and quantitatively and all the necessary amplitude measurements which interest the obstetrician can be obtained. It has been shown that the intensity and duration of uterine contraction and uterine tone increases, while the intervals decrease as labour progresses. The activity of the woman in labour who frequently changes her position accelerates labour by increasing uterine tone and the frequency of contractions.

The understanding of uterine activity and the possibility of its regulation remains one of the most urgent problems of obstetrics. The most important aspect of this problem is to obtain information about the qualitative and quantitative characteristics of uterine contractions during the three stages of labour.

The widely used method of external tocography in spite of its harmlessness, has a number of drawbacks: 1) it is not suitable for making prolonged observations, 2) it restricts the mobility of the patient, 3) it provides predominantly qualitative and not quantitative information. The Alvarez-Caldeyro-Barcia transabdominal method of recording intra-uterine pressure is of high accuracy but due to its complexity it is doubtful whether it can be widely used. The transcervical methods of investigating intra-uterine pressure by means of introducing cylinders (Shatz) or cannulae (Williams, Stallworthy) require that the pregnant woman lies still, and there is a risk of intra-uterine infection.

The search for new methods, in which the above-mentioned disadvantages are eliminated, led to the application of radiotelemetry to the study of uterine activity.

MATERIAL AND METHODS

The principle of transmitting physiological information by radio was first utilized by the Russian scientists A. A. Yuschenko and L. A. Chernavkin (1932) in their studies of saliva secretion in dogs.

Any radiotelemetric system consists of a radio transmitter, which is placed on the surface of or inside of the body and which changes the frequency of its waves under the influence of some function of the body and transmits the waves to a radio receiver. The signals received by the radio receiver may be recorded in different ways on rolls of photographic film or paper.

Within the last few years much information has been accumulated on the application of radiotelemetry in different spheres of biology and medicine, information on the biopotentials of the heart, brain, arterial pulse, respiration, body temperature, blood pressure etc. are transmitted by radio. There have been reports also on the application of this method in obstetrics and gynaecology. Thus, Smith & Wolf (1960) used radiotelemetry for recording intra-uterine pressure and the fetal heart beat in three women; Baughan, Cotes and Parsons (1966) reported on the intra-uterine pressure in monkeys.

In the Soviet Union radiotelemetry has been used since 1964 by S. N. Davydov for studying the activity of the pregnant and nonpregnant uterus. Using this method, the collaborators of S. N. Davydov and L. S. Persianinov carried out numerous investigations of intra-uterine pressure during the three stages of labour (V. T. Aleshker, M. Y. Blok, Y. M. Karash, L. V. Krasnova, K. Kh. Tatevosyan, E. A. Chernukha, C. M. Kochora, B. M. Gasheva & A. Abdukadova), and studied the influence of the complications of labour, the position of labouring woman, drugs, narcosis etc. upon intra-uterine pressure fluctuation.

We use a Soviet-made radiotelemetric "Capsule" device, the 8 × 14 mm cylinder transmitter of which, after treatment with an antiseptic solution, is introduced through the cervix uteri between the membranes and uterine wall before the membranes have ruptured, or beyond the

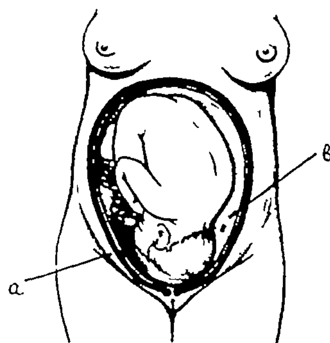


Fig 1 Diagram of position of capsule in cases of intact ( ) and ruptured (*b*) fetal membranes.

presentation part (intra-amniotically) after th membranes ha e ruptured. The radio-sonde (miniature radiostation) introduced in this way is capable of sending information concerning changes in intra-uterine pressure ranging from 0 to 200 mmHg continuously for 7? hours. The precision f the data obtained is guaranteed by a preliminary calibration of the radio-sonde by means of a special gauge.

The radio signals are picked p by the receiver and then are converted into tracing ink recorder

Numerous investigations with simultaneous electrophonocardiography of the fetus and long-term follow-up f the child and mother ha shown that the method is absolutely harmless.

During the first stage of labour the intensity of the contractions is recorded and during the second stage both the in ensity of uterine and luntary muscle contractions I r.e to record this stage contractions, we modified the Mour method by using polyethylene tube, to one end of which a needle as fixed, and the radio-sonde was inserted into the other end. Th tube is filled with sodium citrate Immediately after childbirth the needle of the device is introduced into the umbilical ein, and recording of the intra-uterine (intr placental) pressure can start immediately

Thus, we obtain information about uterine tone and the in ensity and duration of contractions in all three stages of labour Meanwhile the mother may be lying in bed, sitting, walking about the ward, sleeping or under going some obstetrical procedure.

At present we ha e t our disposal data on the radiotelemetric investigation of labour in the follow g conditions:

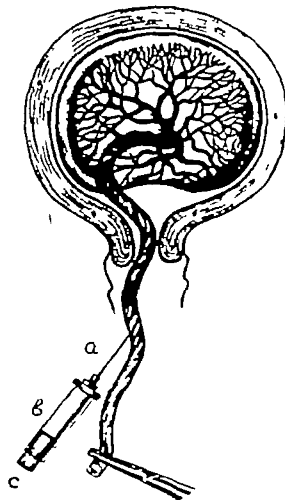
Table I

Type of case	No. of cases
Normal delivery of cephalic presentation	110
Delivery of breech presentation	80
Hypotonic or incoordinate uterine action	170
Rapid or precipitate delivery	55
Extragenital complications of labour	141
Isolated third stage of labour	346
Total	904

RESULTS

The present paper sums up the intra-uterine pressure results in cases of the normal delivery of a cephalic presentation of the fetus.

Continuous radiotelemetric investigation of the



F g Device for recording uterine contractions in the third stage (*a*) needle introduced into the umbilical vein, (*b*) tube filled with sodium citrate (*c*) radio-sonde

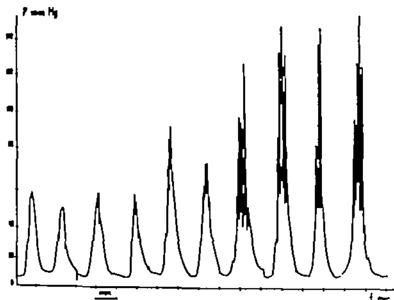


Fig. 3. Radio-tocogram of the first and second stages of labour.

uterine tone revealed that, during the first stage of labour it gradually and slowly increases by approximately 1 mmHg per hour of labour and by the end of the first stage it is twice the initial level. Moreover in primigravidas the tone is somewhat lower than in women giving birth to a subsequent child. Thus, in women having their first child, when the cervix opened to 2–3 cm, the basal tone was 5.3 ± 2.34 mmHg, at 8–10 cm it was 12.0 ± 3.6 mmHg while in multigravidas the tone was $7.16 + 3.12$ and 14.6 ± 3.54 mmHg respectively at the same stages of labour.

During the second stage of labour the uterine tone in primigravidas is 20.7 ± 42 mmHg and 21.96 ± 4.74 mmHg in multigravidas.

In the third stage of labour the basal tone reverts to levels similar t those recorded in the first stage.

The intensity of uterine contractions (that is, the increase in pressure above the basal tone) increases with the progress of labour. Thus, in cases of first delivery when the cervix opened to 2 cm, the intensity was 18 ± 2.34 mmHg, at 8–10 cm dilatation it was 35.0 ± 4.56, and in second and subsequent deliveries it was 19.4 ± 3.21 and 39.4 ± 5.25 respectively at the same stages of labour. During second stage contractions the intensity remains the same as at the end of the first stage, but when the head appears at the vulva, the intensity decreases. During the third stage, until placental separation occurs, the intensity is a little higher than at the end of the first stage of labour.

Expulsive contractions (using voluntary muscles) caused changes in intra-uterine pressure, depending upon the station of the head, at the

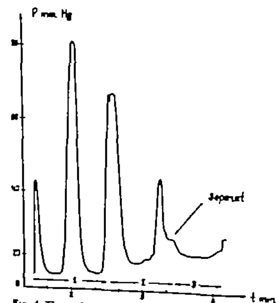


Fig. 4. Three phases of the third stage of labour.



Fig 5 Radio-tocogram, when mcber changes position.

beginning of the second stage in primigravidas, the force was 105 ± 8 4 mmHg, in multigravidas it was 94 4 ± 6.2 mmHg and at the "crowning" of the head 82.5 ± 8.28 and 64 4 ± 16.4 mmHg respectively

The duration of contractions and the intervals between them are shown in Table II (in seconds)

Analysis of this table shows an interrelationship between the duration of uterine contractions and the intervals between them. As labour proceeds, the duration of contractions increases, and the interval decreases. There are no noticeable differences in this respect with parity

Investigation of the characteristics of activity in the third stage of labour has shown that this stage can be divided into 3 phases: I before separation of the placenta, when the contractions follow one another and the configuration of the curve does not change from that of labour II, the process of placental separation itself characterized by the appearance of peaks and drops upon the curve and III the process of expulsion of the placenta, characterized by low amplitude contractions.

Table II *Duration of contractions and intervals (sec)*

	Stages of labour			
	Stage I			Stage III (bef re placental separation)
	dilatation cm	dilatation 8 10 cm	Stage II	
Primigravidas				
Contraction	77 ± 9	88 ± 6	8 ± 5 8	93.5 ± 5 1
Interval	95 ± 25 5	74 ± 19 5	43 6 ± 7 4	61 ± 20 7
Multigravidas				
Contraction	73 7 ± 8 16	87 9 ± 6	85 ± 7 4	9 4 4
Interval	114.5 ± 6.4	85 ± 18 45	45 ± 9 4	4 17 9

A direct relationship between the duration of the first two phases and the volume of blood lost has been noted. In addition the influence of the intensity and duration of uterine contractions on the blood loss in the third stage has been shown.

The changes in uterine activity with varying positions of the mother have been studied in 32 women. It was found that changes in the same horizontal position (mother staying on her back or on her side) changed uterine tone very little (by 2–4 mmHg) and for short periods of time (5–10 minutes) and then the tone reverted to its initial value However when the mother changed her position from supine to either side corresponding to position of the face of the fetus, the intensity of contractions increased by approximately 25% and remained so for a long time.

A change in the mother's position from horizontal to vertical (standing, sitting and walking) at first increased uterine tone by approximately 15 mmHg, but when the change of position was repeated, the effect was diminished The intensity and duration of uterine contractions was only briefly changed when the mother assumed a vertical position

DISCUSSION

A microminiature radio-capsule when in the uterus, gives continuous information about intra-uterine pressure hanges and, thereby about labour without any harm to the mother and fetus.

The increase in uterine tone shown to occur in multigravidas is in disagreement with the data of S R M Reynolds, A I Petchenko 1958 and R Caldeyro-Barcia 1958 who considered that ute

rine tone in primigravidas is higher than in women who have already given birth to a child. However if we take into consideration that the uterus of parous women differs from the uterus of nulliparous women in having greater muscle mass, it is quite reasonable to expect that uterine tonus, which depends on the mass of muscle, will also be higher in a woman who has borne children.

The data obtained on uterine tone in the first stage of labour are, on the whole, identical with the data obtained by Alvarez & Caldeyro-Barcia.

We obtained a direct relationship between uterine tone, contraction intensity duration of contractions and the intervals between them. The higher the frequency of uterine contractions, the greater the tone. The cause–effect relationships here are not quite clear. Is it the increase in tone that leads to the increased frequency of contractions, or the increase in the number of contractions, that leads to the rise in ton ? These questions require further investigation.

We also obtained higher values for voluntary expulsive contractions during the second stage of labour and higher values for tone, than were found by Alvarez & Caldeyro-Barcia.

Apparently the peculiarities of our method made it possible to obtain more reliable data.

The restriction of movement imposed on the mother by the methods formerly used for investigating uterine ctivity led to contradictory data, and to differet recommendations about the best position for the mother. According to our data either a frequent change of position, or the lateral position (with the mother on the same side s the fetal spine), are capable of increasing the intensity of contractions.

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BENIGN GYNAECOLOGICAL TUMOURS AND RADIOISOTOPE RENOGRAPHY

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From the Department of Obstetrics and Gynaecology (Head: Professor P. A. Järvinen), and the Isotope Department (Head: Professor P. J. Taskinen), University of Oulu, Oulu, Finland

Abstract. A series of 91 patients with various benign gynaecological tumours is presented. There were 51 myomas, 21 ovarian tumours, and 19 cases of endometriosis etc. Pre-operatively the ureteral evacuation was disturbed in 29 of the 91 patients (i.e. 32%). The dysfunction disappeared after the operation.

It has long been known that benign gynaecological tumours may produce ureteral dysfunction (4, 5, 11, 13, 17, 18, 19). Isotope renography, on the other hand, has been described as a fast and reliable method, more sensitive than the earlier methods, for examining renal and ureteral function (3, 12, 20, 21). Since isotope renography has been recommended as a routine pre- and post-operative examination of ureteral function (2, 6, 7), it was considered useful to study how various benign gynaecological tumours affected the renogram. At the same time changes following operative therapy were observed.

MATERIAL AND METHOD

Isotope renography was carried out using I^{131}-labelled Hippuran, with the patient in a supine position. Changes in the function of both kidneys were recorded for 15–30 min (9).

Many authors consider qualitative appraisal sufficient in the interpretation of the renography curve (7, 10, 20). In the present study in addition to a qualitative appraisal, half-time was determined. It refers to the time interval between the injection time and descending limb of the curve measured at a level equalling half the maximum height reached (Fig. 2). Arm Winkel (22) considers a half time not exceeding 18 min as normal.

The material comprised an unselected series of 91 patients with benign gynaecological tumours. According to earlier reports (4, 11, 14), ureteral function varies according to whether the tumour is large or small. Tumour sizes have therefore been specified in the data. A tumour larger than the size of two fists was classified as large. The material also covered a few cases of endometriosis and chronic salpingo-oophoritis, with tumour-like formations. Admittedly ureteral dysfunction without tumours has been reported in these conditions (13, 16). According to the principal diagnosis, the material was classified as shown in Table I. Four parovarian cysts were referred to as ovarian tumours. All but 4 patients were operated on. The types of operation ranged from various conservative operations to abdominal hysterectomy. A post-operative renogram was taken on the 5th to 10th day and if this renogram was pathological, a control renogram was taken up to 12 months later. If required, several control renograms were taken.

RESULTS

The results are presented in Table I.

Pre-operatively 29 of the 91 patients (i.e. 32%) were found to have some ureteral dysfunction. Thirteen of the unilateral changes were on the right and 6 on the left side. Seven patients presented a picture of complete ureteral occlusion in their renograms (Fig. 1). Five of these had a large myoma, one had endometriosis and one a large ovarian tumour equal in size to the uterus in the tenth month of gestation. All patients had normal blood creatinine levels.

Six patients with ovarian tumours showed changes, 4 of them unilateral. Where the changes were on the left side the tumour was also on the left. But the 2 patients with changes on the right side had the tumour in the left ovary.

The post-operative renogram was normal in 22 of the 29 patients in whom it had been pathological pre-operatively (Figs. 1 and 2). None of the operated patients showed complete occlusion. On the other hand, 2 patients with a pre-operatively normal renogram showed a pathological

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The post-operative renogram was normal in 22 of the 29 patients in whom it had been pathological pre-operatively (Figs. 1 and). None of the operated patients showed complete occlusion. On the other hand, 2 patients with a pre-operatively normal renogram showed a pathological

Table I

	No.	Operated	Renogram: Pre-operatively pathological: left	right	both	Post-operatively repaired	Pre-operatively normal	Post-operatively pathological: left	right	both
Myoma, small	[illegible]6	6	1	6	5	9	14	0	0	0
large	[illegible]5	[illegible]1	3	-		6	13[a]	0	1	0
Ovarian tumour	[illegible]1	1	-		-	5	15	0	0	1[d]
Endometriosis	14	14[c]	0	[illegible]	1	1	11	0	0	0
Chronic salpingo-oophoritis	5	5	0	1	0	1	4	0	0	0
Total	91	87	6	13	10	22	62	0	1	1
				29						

One not operated.
[a] Three not operated.
One patient with no post-operative renogram (allergy to Hippuran).
[d] Renogram had a technical error. Urography and creatinine were normal.

post-operative renogram. One of them underwent urography with normal results. Unfortunately we were unable to obtain a control renogram but the only curve available post-operatively showed bilateral low maximum activities. This suggests a technical error rather than a poorly excreting kidney (Fig. 3). This patient's creatinine was normal. In the other pathological post-operative renogram the curve showed a quite normal shape but a slightly deflective half-time (Fig. 4).

5 of the 7 patients with a picture of complete ureteral occlusion returned to normal postoperatively. One patient with myoma retained a slightly deflective half-time while the curve had a normal shape. Another with endometriosis could not be re-examined post-operatively owing to an allergic reaction attributed to Hippuran.

DISCUSSION

The influence of benign gynaecological tumours on ureteral function has earlier been studied mainly by means of urography (11, 13, 14, 1[illegible] 18, 19). Series examined by renography have also been reported (2, 6, 8, 15) but they are difficult to compare with the present study since they contained malignant diseases as well, were unspecified, and some were very small.

Urography has disclosed ureteral obstructions

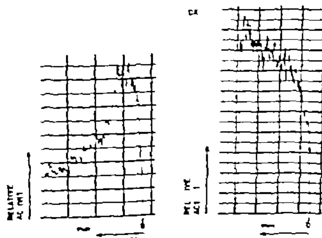


Fig. 1. Pre-operative renogram of patient with large fibroids. In the right renogram the changes typical of total ureteral obstruction are seen.

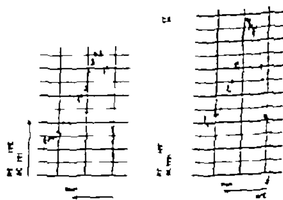


Fig. Post-operative renogram where the changes of Fig. 1 have disappeared and both sides are normal. The half-time of the renogram is indicated with dotted line.

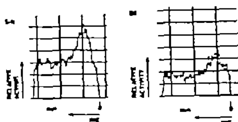


Fig. 3. A post-operative renogram with obvious technical error on both sides. The urography and blood creatinine levels of this patient are normal.

of varying degree in 14.5 to 64.7% of the patients (11, 13, 14, 17, 18, 19). The incidences reported therefore differed widely apparently owing to the varying types of tumour included and the smallness of the series. The largest series, of 379 patients, was that published by Long & Montgomery (13). They reported an incidence of 49.4% of ureteral obstructions, but the series also contained cases of uterine prolapse and malignant tumour. The present pre-operative figure for disturbed emptying of kidneys, 29 of 91 patients (32%), strikes the approximate median for the series examined by urography. Some authors (11, 14) state that a large sized tumour decisively increases the possibility of ureteral obstruction. The same authors find that this tendency is higher in large ovarian tumours than in myomas. This is attributed to the softer consistency of the ovarian tumour which is therefore pushed more tightly against the pelvic brim and causes compression. In the present series also, the large size of the tumour seemed to be a factor predisposing to ureteral obstruction. However, when the patient is in a supine position, as she was during renography, the tumour may compress the ureters more than when she is standing. Neither was the patient's position altered during the examination even if changes suggestive of ureteral obstruction were noted. However, according to Ball (1), the so-called ureteral disturbances can be eliminated by making the patient sit for a while during the examination. zum Winkel (22) gave his patients a spasmolytic; Buscopan® before the examination for the same purpose, but the present patients had no premedication. Therefore, changes suggestive of a complete occlusion must be interpreted with reserve. No conclusions can be drawn concerning the difference in changes produced by large ovarian tumours and large myomas in the present series, which was very small. Long & Montgomery (13) and Widholm et al. (19) reported that the changes were more frequent on the right side. The present results corroborate this finding.

According to Long & Montgomery (13), changes produced by ovarian tumours are usually on the same side as the tumour and only sometimes on the contralateral side. Two of the present patients represented the latter type. One of them was of particular interest in that her right-side adnexa had earlier been removed for ectopic pregnancy. This time the patient had a left-side pseudomucinous cyst, weight 4 600 g, extending to the costal margin, while the renogram showed a deflection of the half-time on the right side. Although it is apparent that the slightly pathological renogram was caused by the large tumour, one may also speculate that the large tumour, owing to its size, has risen out of the true pelvis and therefore did not compress the ureter. If so, the pathologic renogram might have been due to the earlier salpingo-oophorectomy.

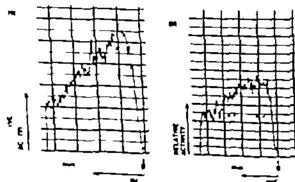


Fig. 4. A slightly pathological post-operative renogram showing quite normal shape, but the half-time of the right side is too long (20 min).

The morphological changes reported in most of the earlier papers were reversible (11 13 14 17) The ureteral function of the patients examined by renography also returned to normal post-operatively

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LOW FIBRINOLYTIC ACTIVITY OF VEINS DURING TREATMENT WITH ETHINYLO ESTRADIOL

Birger Åstedt

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Abstract. Treatment of post-menopausal omen with ethinyloestradiol before operation for prolapse in dose of 250 micrograms day for 10 days significantly suppressed the fibrinolytic activity—determined histochemically—in the vein walls. It enhanced the spontaneous fibrinolytic activity in the blood, and the response of the local fibrinolytic activity of the blood to venous occlusion of the arms tended to increase. These findings may be explained by the assumption that ethinyloestradiol inhibits the synthesis of activators of fibrinolysis or stimulates their release to rate exceeding that of their production. Each alternative ould result in diminution of the activator content of the vessel walls.

Oestrogenic hormones used in conventional combined contraceptives (Grant, 1969 Inman et al. 1970 McQueen, 1970) and given to suppress lactation (Daniel et al 1967 Jeffcoate et al. 1968) or for lowering the blood cholesterol (Oliver 1967) are said to be capable of predisposing to thrombosis. Only a few studies are available on coagulation and fibrinolysis during treatment with oestrogens (Bennett et al 1966 Hampton, 1970 Howie et al 1970) and deal only with changes in the blood.

Changes in the vessel walls have long been thought to play role in the pathogenesis of thrombosis. I recent years it has been shown that the endothelium of certain essels contains activators of the fibrinolytic system (Todd, 1959) and that their release into the blood stream may be enhanced by certain stimuli (Nilsson & Pandolfi, 1970) There is a relationship between the fibrinolytic activity in the vessel walls and the occurrence of thrombosis. Thus, Pandolfi et al. (1967) showed that the fibrinolytic activity in the veins of the lower leg, where thrombosis is much more common, is lower than that in the arms. Isacson & Nilsson (1971) found the local fibrinolytic activity in the vessel walls to be low in 55% of a large series of patients with thrombosis.

This paper reports a study of the fibrinolytic activity in the vein walls during administration of ethinyloestradiol and the liberation of the fibrinolytic agents to the blood on stimulation by venous occlusion. The coagulation factors and the components of the fibrinolytic system were also determined.

MATERIAL AND METHODS

The clinical material consisted of 30 postmenopausal women, who were to have operations for prolapse, but ho were otherwise healthy They received 250 micrograms of ethinyloestradiol day for ten days. Blood samples and biopsy specimens from superficial veins were taken before and during the last day of treatment. Biopsy specimens were taken from 20 of the patients.

Coagulation studies. The following determinations were made: platelet count and platelet adhesiveness in citrated hole blood, fibrinogen (blood collected with EACA), prothrombin + factor VII + factor X (Owren' P & P test), factor V and factor VIII. The methods described previously (Nilsson & Robertson, 1968) were used for the determinations.

Fibrinolytic studies The following determinations were made: fibrinolytic activity of plasma and resuspended euglobulin precipitate on unheated fibrin plates expressed in square millimetres of lysis, plasminogen (clot method), inhibitors of plasminogen activation by urokinase (urokinase inhibitors), α_2-macroglobulin and fibrinolytic split products (blood collected with EACA). The methods are described previously (Nilsson & Robertson, 1968· Ekelund et al., 1970).

Table I *Mean coagulation values before and after administration of ethinyloestradiol for 10 days*

	Before	After
Platelet adhesiveness, %	31	31
Fibrinogen, g/100 ml	0.36	0.42
P&P (factor II, VII X), %	104	107
Factor V %	99	99
Factor VIII %	133	142

$p < 0.01$

Venous stasis was induced by placing a sphygmomanometer cuff around each upper arm inflating it to a pressure between the systolic and the diastolic blood pressure for 20 min. Blood samples for determination of the fibrinolytic activity were collected from each arm before stasis and immediately before the cuff was deflated. The samples were drawn from an antecubital vein. The mean value of the fibrinolytic activity of resuspended euglobulin precipitate after venous stasis found at our laboratory is 280 mm² with a standard deviation of 129 (Robertson, Pandolfi and Nilsson, unpublished data).

Fibrinolytic activity of the vein wall Under local anaesthesia (0.5% Carbocain) a segment from a hand vein was excised. The veins were examined by the fibrinolysis autography technique of Todd (1959) as modified and graded by Pandolfi et al. (1967 1968) Four fibrin slides were prepared for each specimen and incubated at 30°C for 0 5 10, 20 min respectively Three fairly distinct grades of fibrin digestion were recognised, namely grade I. microscopical punctate areas of lysis in most of the sections, grade II. gross lytic areas of irregular outline and sometimes confluent grade III. all or almost all of the fibrin in contact with the sections lysed. The total number of points scored by the set of four slides was taken as measure of the fibrinolytic activity of the sample.

Statistical methods. Comparisons between the individual groups were made by Students *t*-test. The difference of the fibrinolytic activity of the veins was analysed by the Wilcoxon rank sum test.

Table II *Mean values for fibrinolytic components before and after administration of ethinyloestradiol for 10 days*

	Before	After
Plasminogen *	98	124
Urokinase inhibitors, %	125	90
α_2-macroglobulin, %	107	113
Spontaneous fibrinolytic activity on unheated fibrin plates Resusp. euglob. prec., mm	17	50
Fibrinolytic activity on unheated fibrin plates during venous occlusion Resusp. euglob prec., mm²	286	336

$p < 0.05$ $p < 0.01$

RESULTS

The fibrinogen rose significantly from 0.36 to 0.42 g/100 ml ($p < 0.01$) during treatment with ethinyloestradiol. The platelet count and platelet adhesiveness, P & P factor V and factor VIII remained unchanged (Table I). Of the components belonging to the fibrinolytic system, plasminogen increased significantly from 98 to 124% ($p < 0.01$) The inhibitors of urokinase decreased significantly from 125 to 90% ($p < 0.01$) α_2-macroglobulin persisted unchanged (Table II).

The spontaneous fibrinolytic activity increased from 17 to 50 mm² (Table II) and traces of fibrinolytic degradation products appeared during treatment of 10 of the 30 patients. The normal increase of the fibrinolytic activity during stimulation with venous occlusion of the arms tended to rise during treatment (Table II) (Fig 1).

The use of ethinyloestradiol was accompanied by a highly significant decrease ($p < 0.001$) of the fibrinolytic activator content of the vein walls from a mean of 7.5 (range 5.0–10.5) to 5.5 (range 3.5–8.0) arbitrary units (Fig 2)

DISCUSSION

The only change found in the coagulation factors studied was a significant increase of the fibrinogen. This observation is compatible with the findings by Howie et al. (1970) following administration of mestranol (Mestranol®) in a dose of 50 micrograms a day from the 5th to the 25th day of the menstrual cycle. Phillips et al (1961) also reported an increase of the fibrinogen following administration of conjugated equine oestrogen in large doses. Bennett et al. (1966) however found oestriol to lower the fibrinogen

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essentially one and the same method. The inhibitors of urokinase-induced fibrinolysis were significantly decreased. The increase of α_2-macroglobulin reported by Howie et al. (1970) could not be confirmed in our series.

The platelet adhesiveness was unaffected. Neither did Hampton (1970) who used ethinyl-oestradiol but in a dose of 0.05 mg daily find any such change. Bennett et al. (1966) reported slight, but not significant, increase.

The differences between the results may be due to differences in the preparations and doses used.

The suppression of the plasminogen activator activity of the venous wall was highly significant. A hormonal effect on the plasminogen activator content of the vessel wall has also been described by Isacson (1970) who—using the same method—found prednisolone to produce a slight but significant reduction of the activator content. Burke (1969) found administration of oral contraceptives containing oestrogen to raise both the protein bound and non-protein-bound plasma cortisol and that these increases vary with the dose of oestrogen. One might imagine an ffect of ethinyloestradiol is that of cortisol. The marked suppressive ffect of ethinyloestradiol on plasminogen activator activity compared with the mild influence of prednisolone (Isacson, 1970) however suggests a direct effect of ethinyloestradiol on the synthesis and/or release of plasminogen activator

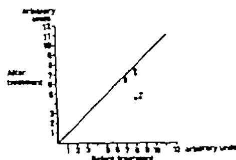


Fig. 2. Fibrinolytic activity of hand veins before and after treatment with ethinyloestradiol for 10 days (histochemically determined. Arbitrary units).

The spontaneous fibrinolytic activity of the blood increased during treatment and degradation products of fibrin appeared in one third of the patients, which suggests an increased release of the fibrinolytic enzyme from the vessel walls. Also the response of the local fibrinolytic activity to venous occlusion of the arms tended to increase. The reduced content of activators of plasminogen in the vessel walls suggests that the synthesis of activators is suppressed or unable to cope with the increased spontaneous release.—Both alternatives would result in diminution of the activity of the vessel wall

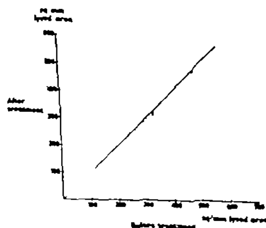


Fig. 1. Fibrinolytic activity of venous blood before and after treatment with ethinyloestradiol for 10 days (resuspended euglobulin precipitate. Sq mm of lysis on fibrin plates).

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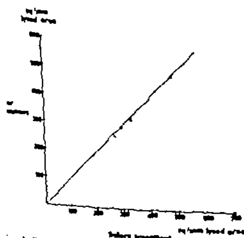


Fig. 1 Fibrinolytic activity of venous blood before and
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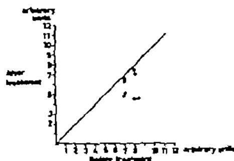


Fig. 2 Fibrinolytic activity of hand veins before and after
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the frequency of thrombosis. Preparations with a high oestrogen content have been held responsible for this increase (Inman et al. 1970· Grant, 1970 McQueen 1970) Daniel et al. (1967) and Jeffcoate et al. (1968) found the frequency of thrombosis during the puerperium to be still higher in women given oestrogenic preparations to suppress lactation. Oliver (1967) reported an increase of thrombotic conditions in men receiving ethinyloestradiol to lower their blood cholesterol level. Treatment of prostatic cancer carcinoma with oestrogenic hormones raises the mortality from cardiovascular diseases (Bailar 1967) In addition oestrogenic hormones have also been reported to have a haemostatic effect (for ref see Andersson, 1962 and Poller 1970)

Against this background the reduction of the fibrinolytic activity of the vein walls during treatment with ethinyloestradiol is interesting. The finding suggests that the hormone may predispose to thrombosis. It should, however be pointed out that no conclusion can be made concerning the significance of the amount of ethinyloestradiol in hormonal contraceptives, which is much smaller On the other hand, the wisdom of unnecessarily heavy treatment of postmenopausal women with ethinyloestradiol may be questioned.

ACKNOWLEDGEMENT

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Acta Obstet Gynec Scand 50: 285–290, 1971

COMPARISON BETWEEN NITROUS OXIDE AND METHOXYFLURANE FOR OBSTETRICAL ANALGESIA

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Abstract. A mixture of 50% nitrous oxide and 50% oxygen was compared with methoxyflurane (Penthrane) in analgesic concentrations during labour. Both drugs were self-administered, nitrous oxide by demand flow through face masks, and methoxyflurane through a special inhaler (Analgizer). The patients used both drugs in succession, with an interval of one labour pain between the two drugs, and were then asked which drug they preferred. The order in which the drugs were given, was decided at random.

Of 63 patients significantly more chose nitrous oxide than methoxyflurane. Both drugs gave excellent or good analgesia in 92% of the cases. Side effects in the mother were in the same order of magnitude for the two drugs. No adverse effects could be detected in the children, by clinical examination. The more frequent choice of nitrous oxide is likely to be due to the differences in the inhalation procedure for the two drugs in this trial, rather than to their analgesic properties. Both can be recommended for pain relief during labour.

The ideal drug for pain relief during labour must be safe, it must work instantly, and it should work on demand at intervals. Self-administered inhalation analgesia goes a long way to meet these requirements. The advent of controlled mixtures of nitrous oxide and oxygen was an important step towards safety. This mixture with 50% of each gas gives good analgesia in most patients in labour. Newer techniques with reliable control of pre-set mixtures (Holmdahl, 1970) make nitrous oxide even better suited for use in obstetrics. This gas has no adverse effects on uterine contractions and on the fetus when given in analgesic concentrations (Bonica, 1967).

One of the newer halogenated anaesthetics, methoxyflurane (Penthrane), is also a potent analgesic when inhaled in low concentrations. Its effect on uterine contractions is less well known than that of nitrous oxide. Methoxyflurane passes through the placenta, but clinical and biochemical studies have failed to show any untoward effects on the children of mothers who had used it for analgesia (Marx et al., 1969; Clark et al., 1970). To ensure against overdosage, a special inhaler (Analgizer) has been designed for use during labour (Marx et al., 1969).

An important aspect of medical research today is the comparison of drugs advertised for the same purpose. The present study is a controlled clinical trial, designed to compare the effects of nitrous oxide and methoxyflurane in labour by letting each patient try both drugs in succession. The results of a field trial from Wales, on the same problem but with a different design, were published while our trial was in progress (Rosen et al., 1969).

MATERIAL AND METHODS

Clinical material

Women in established labour with obvious pain were included in the trial, if delivery was expected to be normal. Patients with a history of liver and kidney disease were excluded, due to possible toxic effects on these organs by larger doses of methoxyflurane (Bonica, 1967; Kazança, 1970). The trial was designed as a sequential analysis, and was therefore discontinued as soon as a statistically valid result had been reached. The total number of patients was 63, of whom 39 were primigravidae and 24 primi- or multiparae. None of the patients had a blood pressure higher than 140/90, four had malpresentation or twins, while vaginal operative delivery was performed in nine cases, and caesarean section in one (maternal indication). Other clinical characteristics can be read directly from Tables I, II and III.

The drugs

Nitrous oxide was mixed with oxygen in 50% concentration. This mixture was either ready made (Entonox, Nor

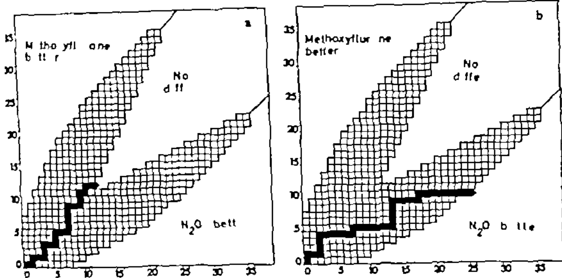


Fig. 1. Results of the trial. (*a*) Nitrous oxide first, 24 patients. (*b*) Methoxyflurane first, 35 patients, plus 1 patient who did not state preference. Level of significance P 0.05

RESULTS

The results of the sequential analysis are given in Fig. 1. There was no difference between the two drugs for those who were given nitrous oxide first (Fig. 1), whereas the patients given the methoxyflurane first chose nitrous oxide significantly more often than methoxyflurane (Fig. 1 *b*), $P <$ 0.05. In Table IV the same results are summarized with three more patients included. Assuming as null hypothesis that the two drugs are equal (expected preference 50 % of each), the observed difference for the total series lies between the 95 % and 99 % confidence limits of the binomial distribution (Documenta Geigy 1962). The implication of this significant preference for nitrous oxide ($0.01 < P < 0.05$) will be discussed.

The degree of analgesic effect was recorded as excellent or good in 58 cases (92 %) and as fair in five cases, the distribution being unrelated to preference (Table V). The objective effect, judged by the doctor corresponded well with the patients' subjective responses. In quite a few cases the patients spontaneously expressed great satisfaction, and some refused to experience another painful contraction pain, as mentioned above. Most of the patients continued to use the drug of preference (33 out of 40 on nitrous oxide and 18 out of 20 on methoxyflurane). The reason for not continuing was as rule that labour had passed into the second stage when the trial was finished.

The need for additional sedative or analgesic medication among the patients who continued to

Table IV. *The drug of preference*

Drug of preference	N_2O first: No of patients	%	Methoxyflurane first: No of patients	%	Total: No of patients	%
Nitrous oxide	1	54	26	70	40	63.5
Methoxyflurane	12	44	10	27	22	35.0
Undecided	0	0	1	3	1	1.5
Total	26	100	37	100	63	100.0

The observed difference for the total series (64.5 % 35.5 % in 62 patients) lies between the 95 % and 99 limits of the exact confidence intervals of the binomial distribution (Documenta Geigy Scientific T bles, 1962).

Table I *Age distribution*

	N O first		Methoxyflurane first	
	No. of patients	%	No of patients	%
Under 20	0	0	4	10.8
20–29	16	61.5	7	73 0
30+	10	38.5	6	16.2

Table II *Parity*

	N_2O first		Methoxyflurane first	
	No. of patients		No. of patients	%
Para 0	16	61.5	23	6..
Para 1+	10	38.5	14	37 8

gas, Norway), or pre-set in an AGA Dedolator (AGA, S eden) (H lmdahl, 1970) In both cases it was inhaled through anaesthetic face masks, working by demand flow

Methoxyflurane (Penthrane, Abbott) was inhaled through the specially made Analgizer (Abbott), which is a cylindrical tube with a mouthpiece. The tube is filled with 15 ml methoxyflurane, which will give up to two hours of analgesia with intermittent breathing. By inhaling through the mouth and exhaling through the nose, the concentration of methoxyflurane vapour is from 0 5% to 0.8 when a diluter hole is covered, and about half that concentration when the hole is open. The Analgizer can be plugged into a face mask, but this was not done in our trial.

In addition to the inhalation analgesics, diazepam and pethidine were also given as required, both before and after the trial. Diazepam was given in doses of 10 mg, and pethidine in doses of 100 mg, both intramuscularly Although it was not part of the trial, such medication was taken into account in the statistical analysis.

The Trial

Each patient tried both nitrous oxide and methoxyflurane, the order of the two drugs being decided at random. A doctor explained the purpose of the trial to the patient, and instructed her in the correct use of the face mask and the inhaler. Inhalation was to start by deep breathing as soon as the contractions were felt, but before the pain developed, and to be discontinued between contractions. The first drug was used for three consecutive contractions, followed by one contraction with air breathing, after which the second drug was used during three contractions. The doctor being present the whole time, then asked the patient which drug was the better one. If she had difficulties in deciding, the question was rephrased thus, "If you were to choose one of them to use from now on, which would you take?" Although the decision often proved difficult, this question was answered by words or by the grabbing of the face mask or the inhaler when the next contraction started. By this approach a positive answer was obtained from all the patients but one.

Including one contraction without analgesia after the first drug had been tried was necessary to ensure minimal residual effect before the next drug. Also, the patients would better be able to appreciate the effects by experiencing this *painful contraction. In at least four patients* this was impossible to carry through, however since they refused to stop inhalation once they had felt its effect. These were therefore immediately switched to the other drug. Another minor deviation from the plan was that one drug was used during two, or four, contractions instead of during three This took place in nine cases. As none of these alterations interfered with the analysis, none of the patients were excluded.

A vaginal examination was performed at the start of the trial and the pulse rate, blood pressure and fetal heart rate were recorded. In a few instances the blood pressure was recorded after the testing of each drug. After the trial, the patients who found relief in the inhalation, continued to use the *drug they preferred.*

Statistical methods

The trial was designed for sequential analysis, slightly modified after Bross (1957). Since the order in which the drugs were given, might influence the preference list of random numbers was consulted to decide the order of the drugs for each patient. This list was kept by office personnel, and was not seen by the doctors. This gave two series of patients, one designed "nitrous oxide first" and the other "methoxyflurane first" For each patient, the preference was plotted into the proper sequential chart. The borderline was not reached simultaneously in both charts. Therefore the total number of patients analysed in the Tables is 63 against 60 in the charts.

In addition to the sequential approach, the material was also analysed with regard to the influence of other clinical parameters, such as the total labour time and the need for additional medication.

To demonstrate the comparability of the two random groups, Tables I, II and III are presented, showing homogeneous distributions of age, parity and cervical dilatation at the start of the trial.

Table III *Dilation of the cervix at the start of the trial*

	N_2O first		Methoxyflurane first	
Dilatation (cm)	No. of patients		No of patients	
1 2	7	6.9	9	4.3
3	7	26 9	10	27 1
4	6	31	10	27 1
5		7 7	3	8.0
6+	4	15 4	4	10.8
Not stated	0	0	1	2.7

ble X. *The trial related to total labour time*

al labour e	Preferred drug N₂O	Methoxy-flurane	Total
6 hours	19	5	24
6 hours	21	17	38
Total	40	22	62

χ^2(5) 0.052

e age of the mother parity dilatation of the ervix at the start of the trial, previous medicaion and attendance t courses for labour preparation. The distribution was homogeneous in every ase, and the drug preference followed the same nain trend in every subgroup. The only slightly kewed distribution was with regard to the duraion of labour (Table X), since markedly more patients with a short labour time preferred nitrous oxide ($P = 0.05$). Considering all the other factors, the most likely explanation is that this represents a chance distribution, and does not indicate biological phenomenon.

DISCUSSION

In a trial of this design it is very important to randomize the order in which the drugs are given. Although the interpretation of the results is more difficult when the end results differ as in the present case, the risk of drawing the wrong conclusion is greater with one than with two groups of patients.

The first difficulty lies in the inherent possibility of the sequential plans of drawing the wrong conclusion (Bross, 1952). In our trial the same question gave two different answers, namely nitrous oxide better and no difference. Assuming that the order in which the drugs are given, is unimportant, the first conclusion is more likely to be correct, as pointed out in the footnote of Table IV. Therefore, we tend to believe that there was a difference, and that nitrous oxide was the better drug. This conclusion applies only to the concentrations and apparatus used in this trial. However the drugs were used in analgesic concentrations, and with apparatus and inhalers specially designed for use in labour.

The difference in choice may be due either to a slight, but consistently better analgesia from nitrous oxide, or it may have been dictated by side effects or difficulties in managing the inhalers, in spite of equal analgesia. In a large field trial of methoxyflurane, nitrous oxide and trichlorethylene in Wales (Rosen et al., 1969) methoxyflurane was found to be sligthly better than nitrous oxide, both apparently inhaled through masks. We believe that the poorer results with methoxyflurane in our trial may well be due to the design of the inhalers, requiring inhalation through the mouth, exhalation through the nose and covering of the diluter hole for maximum relief. A lack of analgetic effect was undoubtedly often due to neglect of these rules. It is quite possible that methoxyflurane inhaled through a face mask might have yielded different results.

Technically breathing through a face mask with a demand flow system was agreeable to most of the patients, although a few were afraid of masks and others complained of mouth dryness.

Ideally all women in labour should have prior knowledge of the apparatus they may use during labour We give instruction in special classes, where the patient can become acquainted with the techniques and be reassured before she is in labour. Such training undoubtedly adds to the beneficial effect of the inhalation analgesics.

A choice of analgesics during labour is an advantage. Pethidine does not act immediately when given intra-muscularly and it may have side effects when given intravenously. The intensity of pain increases steadily during the first stage of labour and additional medication may become necessary at a time when it may harm the fetus. Inhalation analgesics act quickly and can be withdrawn quickly. They can also be given in addition to other drugs. The special design of the inhalation apparatus ensures saf gas concentrations. Other workers have failed to demonstrate any adverse effects on the children f om nitrous oxide/oxygen in analgesic concentrations (review by Bonica, 1967). Methoxyflurane passes the placenta easily and is found in umbilical ein blood in slightly lower concentrations than in maternal venous blood (Clark et al., 1970). The same workers found that when methoxyflurane was used for obstetric analgesia, the blood levels were low and such levels were not associated with

Table V *Pain relief*

	Preferred drug			
	N O	Methoxy flurane	Un-decided	Total
Excellent	3	0	0	3
Good	33	1	1	55
Moderate	4	1	0	5
Poor	0	0	0	0

Table VI *Additional medication after the trial among the patients who continued to use inhalation analgesia*

The probability of a homogeneous distribution regarding "medication < 30 min" calculated against the combination "medication > 30 min" plus no additional drugs is 0.10

Time after start of trial	Preferred drug (no. of patients)	
	N_2O	Methoxyflurane
Diazepam < 30 min	1	1
Diazepam > 30 min	1	4
Pethidine < 30 min	8	0
Pethidine > 30 min	6	3
Diazepam and/or Pethidine < 30 min	9	1
Diazepam and/or Pethidine > 30 min	7	6
No additional drugs	17	12

use the preferred drug, is another measure of the degree of analgesia. This was about the same for both groups (Table VI). Injections were given less often to the methoxyflurane group during the first half hour after the trial, but not significantly so ($P = 0.10$).

Unpleasant subjective side effects are shown in Table VII These were fairly equal for both drugs. Other side effects noted by the observer were drowsiness, also equally distributed light euphoria, only from nitrous oxide hiccups and vomiting (Table VIII). Drowsiness and euphoria are often beneficial side effects. On the other hand, ineffective use of the inhalers is a definite disadvantage Most of the patients used the nitrous oxide demand flow face mask properly after instruction, but at least five did not manage methoxyflurane inhalation, breathing through the nose and not through the inhaler Another methoxyflurane disadvantage was the strong smell which remained in the room for hours.

No trend of blood pressure change was detected for either of the drugs. The course of labour was not influenced by the inhalation analgesia judged by ordinary clinical parameters. There were no perinatal deaths. Fetal heart rate abnormalities were detected during the second stage in four cases, all thought to be unrelated to the drugs. The distribution of Apgar scores is found in Table IX In our experience this distribution must be expected in any group where the mothers are subjected to analgesia in labour

The series was also analysed with regard to

Table VII *Side effects reported by the patients*

As some patients reported more than one side effect, the sums exceed the total number in the trial

	Caused by N_2O	Caused by methoxyflurane
Nausea	4	2
Dizziness, and similar sensations	11	11
Dry mouth, mask unpleasant	6	0
Bad smell or taste	0	9
Numbness	1	0
N reported side effects	42	44

Table VIII *Objective side effects*

	Caused by N_2O	Caused by methoxyflurane
Drowsiness	7	8
Euphoria	4	0
Hiccups	1	0
Vomiting	3	1

Table IX. *Apgar scores*

Mother's preference	Apgar score (no of babies)*						
	4	5	6	7	8	9	10
Preferred and continued to use N_2O	0	1	0		5	1	4
Preferred and cont. to use methoxyflurane	0	1	0	3		1	
Did not continue drug inhalation	0	0	0	0		6	
Total	0		0	5	9	39	8

I the case of twins (scores 7 and 8), 7 has been recorded.

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NEW INSTRUMENTS

A DEVICE FOR MOVING OR CONTROLLING THE POSITION OF THE UTERUS AT LAPAROSCOPY

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A cervical dilatator (Hegar size 6) has been modified by the addition of 0.5 cm wide collar at a distance of 6 cm from the distal end, and by increasing the length of the shaft by 5 cm (Fig 1). The collar is intended to prevent the tip of the instrument from reaching the fundus uteri.

A tenaculum is applied transversely to the anterior lip of the cervix, and then the dilatator is inserted into the uterine cavity Previous dilatation is usually unnecessary The tenaculum and the modified dilatator are taped together (Fig.). Now the uterus can be manipulated freely into any desired position.

As compared with other methods presently in use, the above is at least as effective, and it has the added merit of simplicity

Modification of the Hegar Dilatator as carried out at the hospital' own machine-shop.



Fig 1



Fig

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neonatal depression, although a correlation of higher levels to lower Apgar scores was found. In our experience the distribution of Apgar scores when inhalation analgesia was used is in the range to be expected after the use of any sort of obstetrical analgesia. This is in agreement with the results of Smith & Moya (1968), and of Marx et al. (1969) who found normal values of oxygen tension, carbon dioxide pH and base deficit in the children of mothers who had used methoxyflurane for analgesia.

Both nitrous oxide and methoxyflurane were effective during labour nitrous oxide and oxygen inhaled through face masks on the whole being aligthly more satisfactory than methoxyflurane taken through the special inhaler Provided care is taken over the use of methoxyflurane in liver and kidney disease, and the concomitant prescription of tetracycline (Kuzucu, 1970) both are recommended for use during labour Because of the ease and safety of the apparatus used for inhalation, they can also be used in departments without staff trained in anaesthesia.

ACKNOWLEDGEMENT

Abbott Laboratories, North Chicago Ill. (USA), kindly supplied the methoxyflurane and the Analgizers, f r which due thanks are gi en.

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Table I

Material that con-firmed	Patients															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Total
Parts of decidua	/	/	/		/	/	/	/	/			/		/	/	11
Parts of placenta	/							/	/			/	/	/		6
Parts of membranes	/			/	/	/	/			/	/	/		/		9
Parts of cervix polyp												/				1
Muscle fragments																0

RESULTS

In 4 cases we obtained only blood clots. In 15 cases the evacuated products were subjected to microscopy mainly to determine whether or not fragments of myometrium were present. In no case was muscle tissue obtained (Table I) but it is made possible that some would be found in a larger series.

No complications were observed, with the minor exception of one patient who, although remaining afebrile, developed lower-abdominal tenderness on the third day. Nevertheless, three days late she was symptom-free and fit for discharge from hospital.

CONCLUSION

When weighed against the potential hazards of placental tissue and other products of conception retained in the uterus, the risk of damaging the uterine muscle by post-partum vacuum-evacuation must be negligible. Moreover, as compared with manual removal of the placental remnants, the procedure described here is less of an ordeal for the patient and probably more efficient.

ACKNOWLEDGEMENTS

Microscopic examinations kindly carried out by Dept. of Pathology II, Gothenburg Tes Laboratory (Head: Professor Sig Ranström).

The proposed special suction tube is manufactured by AB Sulte-Werner, Bondegatan 21, S-116 33 Stockholm 4, Sweden.

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A SPECIAL SUCTION TUBE FOR POST PARTUM EVACUATION OF THE UTERUS

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Retained fragments of placenta, membranes and hyperplastic decidua can be effectively removed, after delivery with the proposed special suction-tube

We started with the Nilsson type of suction-tube used for therapeutic abortions and in puerperium We used the largest size No. 14 and added 5 cm in length. To reduce the risk of perforation the instrument was modified by the addition of a 25 mm thick rounded knob surrounding the suction opening, which is 1.5 × 1.8 cm in diameter The leading edge of the suction opening was also modified to reduce curettage to a minimum To facilitate control of the vacuum pressure with the finger our suction-tube has been given a 3 mm wide opening in the holder (Figs. 1 and 2) The vacuum-pressure used does not exceed −0.4 kg/cm² The connection to the suction apparatus is similar to the Nilsson one. Anaesthesia, normally is not needed.

MATERIAL

From April 28th to December 31st 1970 we had 804 deliveries. 19 patients (2.3%) were treated in this way within hours of delivery The indication usually as the suspected retention of small placental fragments, or portions of membrane and, less commonly to control post-partum bleeding from the uterine cavity



Fig 1

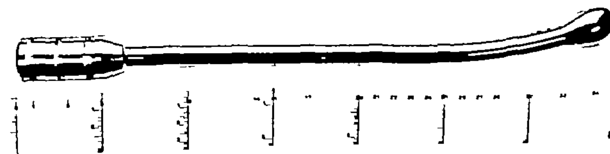


Fig 2

Acta Obstet Gynec Scand 50: 295–300, 1971

CASE REPORT

MARFAN SYNDROME, PREGNANCY AND FATAL DISSECTION OF AORTA

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Abstract. A case of sudden death during early labour is reported from small hospital. The patient had an incomplete Marfan syndrome and death resulted from rupture into the pericardium of dissecting aneurysm of the aorta. No other similar cases ith this combination of the MS, pregnancy and fatal aortic dissection were found among the records of 46 maternal deaths in 75 087 deliveries at two larger hospitals in Southwestern Finland. The case here reported and 12 others previously described showed median age of 25 years at death. The dissection of the aorta, typically ith rupture into the pericardium and tamponade, usually occurred after the 8th month of gestation or in the puerperium. If the pregnancy continued to at least 8 months healthy infant was obtained in most cases. No infants showed signs of the MS. Mostly the anatomical basis of the aortic dissection seemed to be typical cystica medionecrosis ith small partial haemorrhages from the vasa vasorum.

In 1960 McKusick (9) estimated the number of published cases of the Marfan syndrome (MS) to be at least 400, but to our knowledge the actual incidence of this inherited disorder of the connec tiv tissue in the general population is not known. Lately several reports of patients with the MS hose pregnancies ha e terminated f tally due to a dissecting aortic aneurysm (2, 8, 10, 12, 14) ha e focused attention on tall mothers as possible candidates for this unfortunate complication. However although pregnancy is known to be predisposing factor to aortic dissection (5 13) the hos triple complication is rare I the prese t paper case of sudden, unexpected death from ruptured dissecting aneurysm of the aorta of pregnant, tall patient with arachnodactyly is presented and the previous reports of 14 similar cases re briefly reviewed.

CASE HISTORY

This 28-year-old married patient had always been taller than females of similar age. Her daughter also is taller than normal, with long thin legs, and she uses orthopaedic shoes because of looseness of the ankle joints. Otherwise there is no history of similar disorders in the present or previous generation.

The patient had alw y been in a good health and had normal delivery 2 years prior to the present pregnancy. I the present pregnancy the prenatal course had been uneventful. The blood pressure had varied from 120/70 to 130 80 mmHg. Ten days before admission she had complained of intermittent chest pain and simultaneous nausea, especially on exertion. She had not sought medical help for the symptoms and did not even mention of them on admission.

April 4 1965 at 7.30 p.m. the patient was admitted to the District Hospital of Salo, Finland, in the 39th week of gestation. Labour pains had started an hour earlier. A 8.00 p.m. she had regular contractions lasting 2 3 min ith intervals of 3 min. The head of medium sized fetus as engaged at the upper pelvic brim and the external os of the uterus as one finger dilated. No disproportion as detected. The bag of waters had not ruptured and the fetal heart sounds ere strong, 130/min. The patient complained of slight nausea. At 9.30 p.m. the contractions ere as before and the fetal heart sounds 130 min. At 9.45 p.m. the patient as found unconscious and pulseless on the floor. Mouth-to-mouth resuscitation and extrathoracic cardiac massage were started immediately. These therapeutic measures, completed later by endotracheal respiration and electric defibrillation attempts, were not successful. During the early resuscitation procedures the fetal heart sounds became inaudible.

Autopsy

The body height was 188 cm ith long and thin extremities (Table 1). The uterine fundus as located 6 cm below the xiphoid process. Ribs II–V had been broken on the right and II–IV on the left. Each pleural cav y contained approximately 200 ml of serosanguinous fluid, the lungs were oedematous and the liver and spleen slightly congested.

Note

Owing to circumstances that have occurred we beg to inform that the "Northern Association of Obstetricians and Gynaecologists has changed its name into "Scandinavian Association of Obstetricians and Gynaecologists"

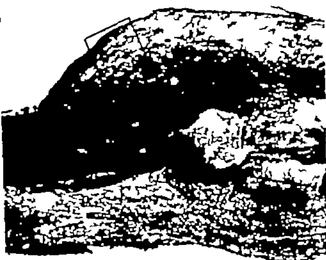


Fig. 2. (*a*) Longitudinal section of ascending aorta. Proximal end of normal thickness to left. Thickening of media with accumulation of amorphous substance mostly in inner layers (upper right) and appearance of large vasa vasorum surrounded by connective tissue in outer layers (right centre). Normal vasa vasorum in adventitia lower left. Hematoxylin-orcein, × 20.



Fig. 2 (*b*) Detail of ... Below normal intima accumulation of ground substance with loss and fragmentation of elastic lamellae. Better preserved elastic tissue lower left. Hematoxylin-orcein, × 100.

We were able to find reports of 14 cases of fatal aortic dissection in pregnant patients with the MS (Table II). But in two of these (12, cases 1 and 14 own case 1) no criteria except the aortic condition are given for the diagnosis of the MS, and these patients simply represent examples of dissecting aneurysm in pregnancy and are excluded from the following discussion. Only two stigmata are described in 3 additional cases (7 10, 11) and in one (12, case 2) the third or ocular manifestation is not ectopia or subluxation of the lens. This leaves 9 patients (including our own) with the definite and 4 with the probable syndrome. Most cases represent an incomplete picture or forme fruste (9).

Incidence

Eleven of the reported cases date from 1965 or later. This is probably due to the greater awareness of the medical profession to the condition. The actual incidence of this fatal combination of the MS, pregnancy and dissecting aneurysm of the aorta remains unknown. Tricomi (14) found 1 case among 74 452 deliveries in New York. We have reviewed the record of all 46 fatal cases among 75 037 deliveries at two hospitals in Turku, Finland (28 179 at the Department of Obstetrics and Gynecology, University of Turku, from 1948 to 1968 and 46 958 at the City Hospital of Turku, from 1945 to 1969) without being able to find a single case in which this fatal syndrome could be suspected. The reported patient comes from

Table I *Some measurements of the patient obtained post mortem*

Height	188 cm	
Vertex to pubis	94 cm	
Pubis to sole	94 cm	
Length of fingers.	Right	Left
I	8 cm	8 cm
II	12 cm	13 cm
III	13.5 cm	14 cm
IV	12 cm	13 cm
V	9.5 cm	9.5 cm

The significant findings were found in the cardiovascular system. The pericardium contained 500 g blood clot and 600 ml of haemorrhagic fluid. The heart weighed 400 g. The cusps of the aortic valve were thin and tw of them showed small fenestrations close to their border (Fig. 1 short arrows). The pulmonary valve cusps were also thin. Immediately above the sinuses of Valsalva, a 4 cm long transverse tear was seen in the intima (Fig. 1 long arrows). This led into a dissection which contained a thrombus and continued approximately 5 cm distally Through the left lateral wall of the ascending aorta there was a rupture into the pericardium. Another small dissec tion with an intimal tear but no external rupture was detected in the abdominal aorta. There was no atherosclerosis and the aortic ring was not dilated.

Microscopically the ascending aorta was thickened due to two processes in the tunica media (Fig. 2). Amorphous material had accumulated, mostly in the inner parts This stained positively with Alcian blue but not with periodic acid-Schiff. In the outer third the vasa vasorum were dilated and surrounded by connective tissue with a slight inflammatory infiltration and an occasional small haemorrhage (Fig. 3). The main dissection was also located in the outer third of the media in the ascending aorta but extended to the inner half in the abdominal aorta. The elastic fibres had disrupted and ere few in number (Fig. 2 *b*). The smooth muscle cells, having lost their support, showed bizarre configurations. Both of these degenerative changes were most abundant in the ascending, less in abdominal and least in thoracic aorta.

The male fetus weighed 3 400 g, was 5 cm long and did not show arachnodactyly The histologic sections of the aorta were normal, but a complete autopsy was not performed on the fetus.

DISCUSSION

Diagnosis

The cardinal manifestations of the Marfan syndrome are long, thin extremities, redundant ligaments and joint capsules, ectopia lentis, and dif fuse dilatation and/or dissection of the aorta (9). The most reliable stigmata are considered to be ec topia lentis and the presence of other unequivocally affected members in the patient s family If neither of these criteria is met, the diagnosis is left in question. In the literature there seems to be some confusion in this respect. E.g. Tricomi (14) has reviewed from the literature 10 cases of death from dissecting aneurysm during pregnancy in patients with the MS However a study of his references shows that in many cases the above diagnostic criteria are lacking.



Fig 1 Thin aortic valve cusps with fenestrations at border (*short arrows*), intimal tear immediately above (*long arrows*).



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Fig. 3. In outer third of aortic media a small haemorrhage with fibrin (arrow) surrounded by hyalinized connective tissue and adjacent to abnormal blood vessel (left centre). Hematoxylin-van Gieson. ×51.

different, although close area of the country the incidence figures of which cannot be calculated. We may conclude that, at least in this part of Finland, the condition is extremely rare. The largest series to date is that of Donaldson & de Alvarez (2) who in addition to the 4 cases included in Table II present 8 other pregnant patients who did not have this fatal complication during pregnancy or the puerperium. The authors give no incidence figures.

Clinical features

The youngest pregnant MS patient with fatal aortic dissection in the literature reviewed was 21

Table II. *Age of patients and diagnostic criteria of Marfan syndrome recorded in 14 cases from literature and in present case*

Author and date of publication		Age of patient	Stigmata of Marfan syndrome described: Ocular: Ectopia lentis	Ocular: Myopia	Musculo-Skeletal	Cardio-vascular	Family history
Lindeboom & Bower (1950)		[illegible]3	no	no	yes	yes	not known
Husebye et al. (1958)		30	no	no	yes	yes	positive
Novell et al. (1958)		4	no	no	yes	yes	negative
Borglin & Bach (1961)		[illegible]3	no	yes	yes	yes	positive?
Donaldson & de Alvarez (1965)	1	24	no	no	yes	yes	positive
	2	[illegible]	no	yes	yes	yes	positive
	3	1	no	no	yes	yes	positive
	4	77	yes	no	yes	yes	positive
Moore (1965)		4	no	no	yes	yes	not known
Tricomi (1965)	1	30	no	no	no	yes	not known
	2	[illegible]5	no	yes	yes	yes	positive
Masumi et al. (1967)		35	yes	no	yes	yes	positive
Rifkin (1967)	1	33	no	no	no	yes	not known
	2	[illegible]3	no	yes	yes	yes	not known
Sutinen & Piiroinen (present case)		[illegible]8		no	yes	yes	positive

Table III *Time of aortic dissection related to pregnancy and fate of infants in 12 cases of Marfan syndrome from literature and in present case*

Author and date of publication	Number of patients									Infants	
	Time of dissection										
	Months of pregnancy										
	IV	V	VI	VII	VIII	IX	X	Puerperium	Total	Living	Dead
Lindeboom & Bower (1950)				1					1		1
Hamby et al. (1958)		1[a]							1	1	
Novell et al. (1958)								1	1	1	
Borghi & Bach (1961)								1	1	1	
Donaldson & de Alvarez (1965)	1		1			1		1	4	2	2
Moore (1965)								1	1	1	
Tricomi (1965)						1			1		1
Mammen et al. (1967)					1				1	1	
Rivlin (1967)					1[c]				1	1	
Sattrum & Parowen (present case)							1		1		1
Total	1	1	1	1	2	2	1	4	13	8	5

Caesarean section 10 days before term, death 10 months postpartum.
Post-mortem caesarean section
Delivery at term, death 8 weeks postpartum.

and the oldest 35 years at the time of death with a median of 25 years (Table II). Three patients were in their first, 5 their second, 3 their third, and 2 their fourth pregnancy. One of the patients had had previous abortion, the other previous pregnancies having been close to full term. In the monograph of McKusick (9) we found descriptions of 41 patients with the MS who died from some cardiovascular complication. Their age at death varied from 6 months to 53 years, with two peaks of incidence at 10–14 and 30–34 years.

Four aortic dissections occurred before the 8th month of gestation, five during the last 3 months and four in the puerperium (Table III). Usually death followed in a few hours or days, except in 2 cases.

Infants

Eight living infants out of 13 pregnancies were obtained (Table III). Four of these were delivered by caesarean section, one post mortem. Three of the five dead fetuses were under 8 months gestational age. None of the living infants showed definite signs of the MS although in one case (1) the great toes were rather long. Autopsy data of the dead fetuses are not available. In our case the fetal aorta was macroscopically and histologically normal.

Pathology gross

Adequate gross description of the aorta is given in 9 cases. Aneurysmal dilatation of the ascending aorta was present in 5 patients and in 2 of these another dilated area was found more distally. In five instances dissection of the ascending aorta continued to the abdominal aorta and in two a separate area of dissection was seen in the abdominal or in the thoracic and abdominal aorta. The major branches of the aortic arch were involved in two cases and the common iliac arteries in another two. The intimal tear usually transverse was located in the proximal part of the ascending aorta in eight cases and in the arch in one. An external rupture into the pericardium with cardiac tamponade occurred six times and once into the posterior mediastinum. In two cases with no external rupture the immediate causes of death were, respectively, congestive heart failure and renal cortical necrosis with anuria. In addition to the present case a fenestrated aortic valve was described by Novell et al. (11). Their patient also had a coarctation.

Microscopic

A histological description of the aorta is available in six cases. In five the basic lesion was typical of Erdheim s (3) cystic medial necrosis with disruption of elastic lamellae and accumulation of amorphous material in the media. In one case (6) the elastic degeneration was not accompanied by an increase of ground substance. The dissection in the lesions described was located in the outer third of the aortic media. Our observations support the theory (4 13) that the dissection starts as a small haemorrhage from the vasa vasorum in the outer layers of the degenerated tunica media which has lost its elastic support.

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PLASMA OESTROGENS AND THE FETAL OUTCOME

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Abstract. Oestriol, oestrone, and oestradiol-17β concentrations in plasma were studied in a series of "high risk" pregnancies. A gas chromatographic method of measurement was used. Plasma determinations have an advantage over urine determinations, because the collection of 24 h urine specimens is tedious and particularly liable to error. Low and subnormal plasma oestriol values were found to be correlated with the subsequent occurrence of fetal asphyxia or intrauterine death. Plasma oestriol values within normal limits, or increasing, indicated that fetuses were not at risk of the more severe grades of asphyxia, or of intrauterine death. An exception was observed when the mothers had impaired renal function. Oestrogen clearance is reduced and, therefore, apparently normal values are not significant. Low oestrogen concentrations may not be significant when patients are on [illegible] steroid therapy. Plasma oestrone and oestradiol-17β values in the asphyxia group showed a similar pattern to those of oestriol, but gave no additional information. In the groups without asphyxia, especially oestradiol-17β values lay low in the normal range with many subnormal values. In many of these cases, there was also the discrepancy that plasma oestriol values were normal, while urine oestriol values were subnormal. The plasma analyses will probably become a valuable guide to care in the obstetrical clinic.

The determination of oestriol in the urine of pregnant women is frequently undertaken in obstetrical clinics. It is a valuable test, but suffers from the disadvantage that 24 h urine specimens are required and considerable cooperation from the patients is necessary. The possibility of error in collection is high, and the collection is inconvenient, especially for out-patients.

These problems have led to the study of oestriol levels in plasma and a consideration of their value in monitoring fetal welfare, additional to, or in place of, urine oestriol determinations. Such studies (18, 22, 23, 27) indicated that plasma oestriol determination is as good a guide in pregnancies complicated by pre-eclampsia, or intrauterine death of the fetus, and Taylor et al. (30) consider that blood analysis is a more reliable parameter of fetal condition than urinalyses.

In late pregnancy oestriol is mainly of fetal origin, the precursor being 16α-hydroxy-dehydroisoandrosterone. To a lesser extent oestriol is derived from placental oestrone and oestradiol-17β. Siiteri & McDonald (28) suggest that the precursor of the latter two is dehydroisoandrosterone equally of fetal and maternal origin. Both oestrone and oestradiol-17β therefore seem to be important metabolites, and the demonstration, when the umbilical cord is ligated in utero (6) and when the fetus is anencephalic, (14), that the urinary excretion of all three oestrogens is low indicates, that oestrone and oestradiol-17β may also be of value in estimating the fetal condition.

This present work is a study of all three oestrogens in plasma, and of oestriol in urine in a series of complicated pregnancies. To identify any prognostic value of such studies, the results are related to the state of child at birth, rather than to any illness in the mother.

METHODS

The author has earlier reported a gas chromatographic method for measurement of oestriol, oestrone and oestradiol-17β in plasma (8, 10). A specimen of 5 ml heparinized plasma obtained from a venous blood sample is required for the analysis. Results are corrected for analysis loss on the basis of the radioactive recovery of tritium labelled oestrogens added as internal standards. If the analysis is restricted to oestriol, only 3 ml plasma is required, and the time of analysis is approximately two working days.

The normal range in μg/litre for oestriol, oestrone and oestradiol-17β in the plasma of pregnant women as determined by a study of [illegible] normal women from the 23rd to 41st week of pregnancy (9, 11, 17). The mean values

Table I *Fifteen cases of intrauterine death and neonatal asphyxia*

Pt. no.	Parity	Pregnancy	Delivery	Sex	Birth weight (g)	Week	Neonatal state (est. Salinq-score)
1	1	Hypertension intrauterine death	Oxytocin induced labour	M	870	3	Stillborn
2	1	Preeclampsia intrauterine death	Oxytocin induced labour	F	1 480	38	Stillborn
3	3	Rh-iso-immunisation, intrauterine death	Oxytocin in labour	F	950	28	Stillborn, hydrops
4	1	Diabetes mellitus, anencephaly intrauterine death	Oxytocin induced labour	F	1 500	37	Stillborn, acrania and anencephaly
5	6	Hypertension placental insufficiency	Caesarean section	M	3 000	38	Poor tone deficient respiration first 5 min
6	1	Preeclampsia	Spontaneous	F	1 910	38	Deficient respiration (9)
7	2	Preeclampsia placental insufficiency	Caesarean section	F	2 300	40	Poor tone ox pre-treatment (10)
8	2	Hyperchromic anaemia, placental insufficiency	Spontaneous	F	[illegible]000	38	Cyanosis, treated by hyperbaric oxygen
9		Chronic pyelonephritis, placental insufficiency	Caesarean section	77	2 150	39	Convulsions during first day (9)
10	1	Small fetus placental insufficiency	Inertia oxytocin in labour	F	2 300	41	Absent tone assisted respiration episodes of cardiac arrest
11	1	Small fetus	Pyrexia in labour	M	150	38	Absent tone jaundice exchange transf (9)
12	1	Placental insufficiency	Oxytocin induced labour after escaped amniotic fluid	M	3 050	40	Absent respiration (9)
13	3	Threatened death of fetus placental insufficiency	Caesarean section (greenish amniotic fluid)	F	1 960	40	Cyanosis, poor tone deficient respiration (8)
14		Placental insufficiency	Escape fo mniotic fluid before labour nd prolonged labour	M	2 800	42	Cyanosis n 4 h oxygen treatment (10)
15	1	Placental insufficiency breech presentation	Inertia oxytoci in labour ncomplete extraction	F	3 170	41	Cyanosis poor tone, deficient respiration (8)

and 95 limits re shown in Figs. 1 3 and 5 by the straight lines.

Urinary oestriol determinations using Frandsen's method (13) were undertaken t Statens Seruminstitut, Copenhagen. The curves for mean values and pper and lower limits in normal pregnancies previously determined by Frandsen (13) are show for reference (Figs. , 4 and 6).

MATERIAL

The 44 patients were all under the care of the department and were all delivered during their admission. I all cases the past history previou xamination in the department indicated the likelihood f obstetrical complications. Often, i was the finding f one or more low urine oestriol lues which led t the study f the plasma oestrogens. In 9 instances onl one blood sample s taken before term.

A total of 173 plasma oestriol, 167 plasma oestrone and 157 plasma oestradiol-17β analyses ere made, predominantly in duplicate. The 178 rinary oestriol determinations show in Figs. , 4 d 6 re those measured for the 4 hours during which blood samples re taken for plasma oestrogen analyses.

Table II. *Nine cases without neonatal asphyxia, but with low birth weight (< 2500 g) at the calculated time of delivery ("Small for date"-babies)*

Pat. no.	Parity	Pregnancy	Delivery	Sex	Birth weight (g)	Week	Neonatal state (evt. Saling-score)
1	2	Placental insufficiency	Oxytocin induced labour after escaped amniotic fluid	F	2 240	40	Normal (12)
2	2	Placental insufficiency	Prolonged labour oxytocin in labour	M	2 300	40	Normal (12)
3	2	Placental insufficiency	Spontaneous	M	2 000	38	Normal (11)
4	2	Placental insufficiency	Spontaneous	M	2 380	40	Normal (12)
5	1	Placental insufficiency	Inertia, oxytocin in labour forceps (fetal brady-cardia)	F	2 300	41	Normal (11)
6	3	Possible placental insufficiency	Prolonged labour oxytocin in labour	F	2 200	41	Normal (12)
7	1	Placental insufficiency	Spontaneous	F	2 150	42	Normal (12)
8	2	Hydrorrhoea	Spontaneous	F	2 250	40	Normal (12)
9	2	Chronic glomerulo-nephritis	Spontaneous	M	2 220	39	Normal (12)

Cases are allotted to one of three groups, according to the status of the newborn babies. The first group of *intrauterine death and neonatal asphyxia*, contains 15 patients. Saling scores are appended. The clinical data is summarized in Table I.

The second group (Table II) contains children who were *not asphyxiated* born at term as calculated from the date of the last period, but with *birth weights* < *2 500 g*. It consists of nine small for dates children, all in good neonatal condition, and not requiring any treatment.

The third group (Table III) of 20 children *not asphyxiated and with birth weights* > *2 500 g*.

Clinical estimations of the condition of the newborn were made without knowledge of the plasma oestrogen values, and analyses were undertaken without knowledge of the obstetrical course and previous history. Tables I, II and III give the relevant diagnoses. If the only sign of abnormality was low urinary oestriol, the diagnosis "placental insufficiency" was made, and if only isolated low values were found, this diagnosis is qualified by "possible placental insufficiency".

RESULTS

Fig. 1 gives the results of plasma oestrogen determinations in the first group (Table I) of intrauterine death and neonatal asphyxia. Single plasma oestriol values were below the normal range or there was a falling curve also reflected by the plasma oestrone and oestradiol-17β determinations, though subnormal values were less frequent.

The urinary oestriol excretion of patients in Table I was generally below normal (Fig.)

Special mention is made of certain patients in Table I. In patient 1 intrauterine death occurred after the penultimate test (Fig. 1). The ultimate test was taken 5 days late at which time the patient had not noticed fetal movement for the preceding 2 days, and she delivered a stillborn baby days later. In patients 2 and 3 fetal heart sounds had not been heard for 1 and 7 days, respectively before plasma oestrogen determinations. They delivered stillborn babies 6 and 3 days later. In patient 4 fetal death probably occurred between the first and second determinations. The last determination was made within 24 hours of the delivery of a dead anencephalic monster. Plasma oestriol values were subnormal in all patients where intrauterine death occurred, but only in patient 2 were plasma oestrone and oestradiol-17β also subnormal.

Eight patients (No 5, 6, 7, 8, 10, 11, 12 and 13 Table I) demonstrate that varying degrees of neonatal asphyxia are associated with low plasma oestriol values, but also that no particular lower

Table III *Twenty cases without neonatal asphyxia and with birth weights > 2500 g*

Pat. no	Parity	Pregnancy	Delivery	Sex	Birth weight (g)	Week	Neonatal state (evt. Saling-score)
1	1	Preeclampsia	Vacuum-extractor	M	3 100	38	Normal (1[illegible])
	1	Hypertension	Spontaneous	F	[illegible] 550	41	Normal (11)
3	3	Hypertension	Oxytocin induced labour after escaped amniotic fluid	M	2 560	38	Pneumonia 3 days old (12)
4	1	Hypertension	Caesarean section (contracted pelvis)	F	3 400	40	Normal (1[illegible])
5	1	Proteinuria, presumed chronic pyelonephritis	Caesarean section (persistent occipito-anterior position)	M	2 750	42	Normal (12)
6	2	Rh-iso-immunosation	Oxytycin induced labour	F	2 710	41	Hemolytic disease no exchange trans. (11)
7	3	Bronchial asthma (betamethasone)	Inertia oxytocin in labour	M	3 200	40	Normal
8	1	Infertility	Caesarean section	M	3 900	42	Normal (12)
9	3	Placental insufficiency	Caesarean section (former child with cerebral palsy)	M	2 900	40	Normal (12)
10	1	Placental insufficiency	Spontaneous	F	2 550	38–39	Normal (11)
11	1	Placental insufficiency, large fetus	Oxytocin induced labour, episiotomy (fetal bradycardia)	M	3 650	41	Normal (1[illegible])
12	1	Placental insufficiency pruritus	Oxytocin induced labour forceps (fetal bradycardia)	M	3 350	42	Normal (1[illegible])
13	1	Placental insufficiency	Spontaneous	M	2 600	41	Died 22 days old of congestive cardiac failure (12)
14	2	Placental insufficiency	Oxytocin induced labour	F	3 150	39	Normal
15	2	Possible placental insufficiency	Inertia oxytocin in labour	F	2 880	41	Normal (12)
16	1	Possible placental insufficiency	Spontaneous	F	3 600	40	Normal (11)
17	1	Possible placental insufficiency	Spontaneous	F	3 750	40	Normal (1[illegible])
18	1	Possible placental insufficiency, large fetus	Oxytocin induced labour	M	4 080	41	Normal (12)
19	1	Possible placental insufficiency	Spontaneous	F	3 075	41	Normal (11)
20	1	Possible placental insufficiency	Spontaneous	F	700	41	Normal (12)

limit can be set as definitely indicative of intra uterine death.

Only one plasma oestrogen determination was made in patient 9 who had chronic pyelonephritis and impaired kidney function. Oestrone and oestradiol-17β were low while oestriol was high in plasma and low in urine. In patient 14 plasma oestriol values, initially subnormal increased to well within normal limits. Oestradiol-17β however was very low. The child had only mild neonatal asphyxia. All three oestrogens showed a similar peak in patient 15 but this was not

reflected in urinary oestriol values. The child was a breech presentation and incomplete extraction was performed. There was some difficulty in delivering the head, which alone could be responsible for the asphyxia.

Plasma oestrogen determinations in the second group of the study (Table II) are shown in Fig. 3. The children had no neonatal asphyxia but were "small for dates". This was also suspected from clinical examinations, and in no instance was there

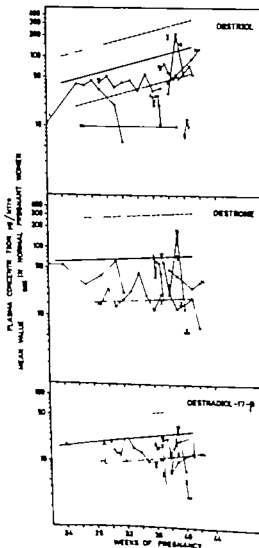


Fig. 1. Plasma oestrogens in 15 cases of intra-uterine death or neonatal asphyxia (Table I). Numbers refer to patients in the table. Logarithmic ordinate. Oestriol N 47; oestrone N 4; oestradiol-17β N

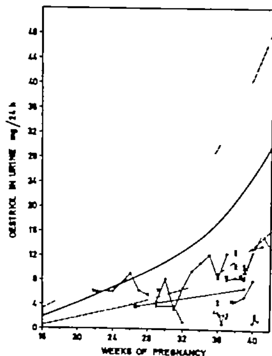


Fig. 2. Urine oestriol in 15 cases of intra-uterine death or neonatal asphyxia (Table I). Numbers refer to patients in the table. Measurements made at the same time as plasma oestrogen determinations (Fig. 1). *N* 47

any ground for suspicion that the gestational age might have been calculated inaccurately. The plasma oestriol values shown in Fig. 3 are mostly within normal limits. In two instances (Patients 1 and 6, Table II) oestriol values were initially subnormal, but subsequently within normal limits. Plasma oestrone and oestradiol-17β values did not conform, but many oestradiol-17β determinations were at or just below the lower limit of normal. Patient 9 had chronic glomerulonephritis, but virtually normal kidney function.

Urinary oestriol determinations in this group (Fig. 4) were in some cases, at levels which are thought to reflect impending fetal death (Patients 1, 3, 4, 5 and 7).

Plasma oestrogen values in the third group (Table III) are shown in Fig. 5. The children had no neonatal asphyxia and birth weigths greater than 2500 g. The vast majority of plasma oestriol determinations lay well within the normal range, as did oestrone, but oestradiol-17β values were generally around the lower limit of normal and several were subnormal.

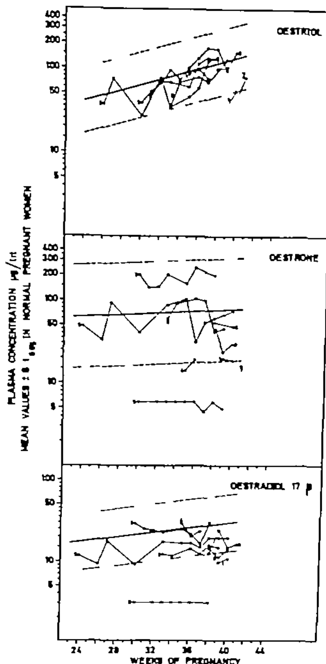


Fig 3 Plasma oestrogens in 9 cases without neonatal asphyxia, but with birth weights ≤ 500 g t the calculated time of delivery (Table II) Numbers refer to patients in the table Logarithmic ordinate. Oestriol. $N = 44$ oestrone: $N = 44$ oestradiol-17β: $N = 45$

Urinary oestriol in the group (Fig. 6) was low in, or under the normal range.

Patient 3 was allotted to the group without neonatal asphyxia despite treatment of the child for pneumonia on the third day after delivery The patient had normal plasma oestrogen and urine oestriol values. The child born to Patient 13 died 22 days after birth from sepsis and congenital heart disease but was not asphyxiated in the neonatal period. Plasma oestrogens were normal, but urinary oestriol was below the normal range.

Patient 7 was treated with betamethazone ("Celeston"®) at the time of study and Patient 14, who also had low oestrogens, had recently been recommended to discontinue thyroxine and various psychotropic drugs. She had a long history of drug consumption and it is not certain that she observed the injunction.

DISCUSSION

Two patients in the asphyxia group (Nos. 9 and 15 Table I) had high plasma oestriol values (Fig. 1) and thus differ from the group characteristic. Patient 9 however had reduced kidney function reflected by serum urea (83 mg) and creatinine values (1 9 mg%) and by creatinine clearance (27 ml/min) The high plasma oestriol values may thus be explained by reduced renal clearance, as also observed by other authors (5 16, 18 26) in uraemic patients. Urinary oestriol excretion was subnormal. Patient 15 had one high plasma oestriol determination, and oestrone and oestra-

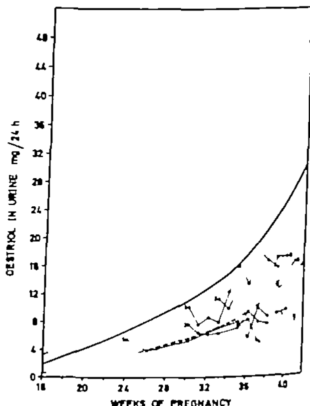


Fig 4 Urine oestriol measurements made at the same time as the plasma oestrogen determinations in Fig 3. $N = 47$

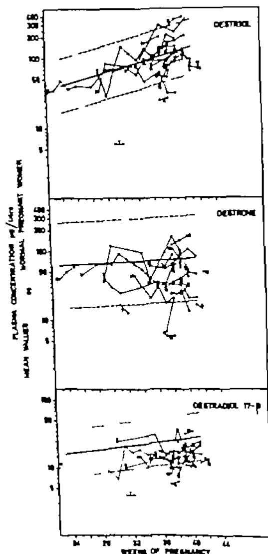


Fig. Plasma oestrogens in 20 cases without neonatal asphyxia and with birth weight 2 500 g (*Table III*). Numbers refer to patients in the table. Logarithmic ordinate. Oestriol N 82, oestrone N 78, oestradiol-17β N 77.

diol-17β w re similarly high. The child was delivered as breech presentation and neonatal sphyxia possibly resulted from the difficulty in deli ering the head, but the other low plasma oestriol lues, and the low urinary oestriol, may ery well indicate that the child was particularly lnerable to the unpredictable complications inherent in ginal deliveries of breech presentations I P tient 14 (Table I) plasma oestriol rose from subnormal to well within normal limits. Oestradiol-17β however was very low. The child was cyanotic for some hours after birth, but otherwise displayed no signs of asphyxia.

Low plasma oestriol values associated with intra-uterine death have been reported by other authors (18, 22, 23, 27), but not all have been under the lower limit of a determined norm. Roy et al. (23) found that plasma oestrone and oestradiol-17β were also low in women with fetal death in utero.

The finding of low plasma oestriol in Patient 4 (Table I) who delivered a dead anencephalic monster and was a diabetic, in whom incidence of fetal malformation is more frequent (20), is in accordance with that of Frandsen & Stakemann (14). They found low urinary oestrogen excretion in women with anencephalic monsters with suprarenal hypoplasia. B rkeland (2) found low urinary oestriol in a case of an infant with a normal head but with bilateral suprarenal aplasia. In the patient discussed here, the fetal suprarenal glands were also found to be hypoplastic. Svendsen & Sørensen (29) found low plasma oestrone and oestradiol-

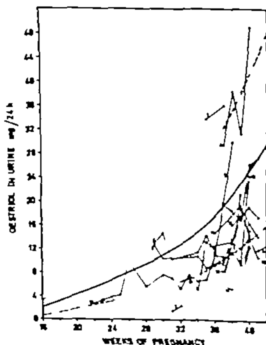


Fig. 4. Urine oestriol measurements made at the same time as the plasma oestrogen determinations in Fig. 5. N 84.

17β concentrations when fetuses were subsequently found to be anencephalic. The last plasma determinations were made within the first 24 hours after the birth of the dead fetus. The fall in oestrogens after delivery of fetus and placenta has also been observed by others, in urine (4) and in plasma (21)

In the group of normal weight children without neonatal asphyxia, two mothers (Patients 7 and 14 Table III) had plasma oestrogen concentrations below normal. Patient 7 was treated with betamethasone, and this has been found to reduce urinary oestrogen excretion to subnormal at the corresponding gestational age (15 Case A and Patient 7 are identical) Apart from any possible differences in transplacental passage this effect seems to be dose dependent, and can be reproduced with non-halogenated corticosteroids in appropriately high doses (3 7 19) The low plasma oestrogen concentration and low urinary excretion in Patient 14 cannot be explained with certainty It may well be that she continued to take various drugs during pregnancy despite contrary instructions. Steroids were probably not among her various medicaments.

Several authors report that blood and urinary oestriol determinations are not of value when there is rhesus incompatibility (17 24 25) Amniotic fluid oestriol concentration, however may be a more reliable indication of the fetal state (1 25) This present study does not refute or confirm these findings. There were two instances of rhesus incompatibility Patient 6 (Table III) had a child with haemolytic disease of the new born, but exchange transfusion was not necessary and there was no neonatal asphyxia. Plasma oestriol was normal. Patient 3 (Table I) had a stillborn hydropic child Only one plasma study was made probably at a time when intra-uterine death had already occurred. Oestrogen concentrations were subnormal (Fig. 1)

To explain the discrepancies between plasma and urinary oestriol greater experience of plasma studies has to be gained. Studies of renal clearance of the different oestriol conjugates present in the blood may be necessary

CONCLUSION

In general, a fall in plasma oestriol concentration to around or below the lower limit of normal indicated that the fetus was at risk. When concentrations lay well within normal limits or showed an increase, no serious degrees of asphyxia were seen.

This relationship between plasma oestriol concentration and fetal distress is not valid when mothers have impaired renal function. Misleading high oestriol values may then be found because renal clearance is reduced. When patients are under (halogenated) steroid therapy low oestriol values do not necessarily indicate a poor fetal prognosis.

In the asphyxia group plasma oestrone and oestradiol-17β patterns were similar to that of oestriol, and apparently gave no additional guidance. In the other groups plasma oestradiol-17β concentrations were often low in the normal range, and often subnormal.

The experience of this study as of others cited is that plasma oestriol determination can become a valuable guide to the control of antenatal patients.

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I should particularly like to thank Dr S and Vestergaard f the University Dept. of Obstetrics and Gynaecology Rigshospitalet, who gave invaluable aid in the collection f this series of patients.

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AETIOLOGY OF POSTMENOPAUSAL BLEEDING

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Abstract. A series consisting of 1 085 women with post menopausal genital bleeding as investigated. The women ere at least 45 years old and the time lapse from the last menstruation as at least one year. The bleeding was due to malignant process in 28%. The majority of these patients had endometrial or cervical carcinoma. The ratio of these two conditions was 1 1. The median age of e patients showing malignant growth was clearly higher an the median age of those in hom the cause of bleedg was benign. The rate of malignant tumours as cause f postmenopausal bleeding showed definite rise ith creasing age.

Atrophic endometrium was the cause of bleeding in 0.5% of the patients. A hormonal reaction in the endometrium as involved in 18.5%, an endometrial or cervial polyp in 17.2%, cervicitis ith erosion in 12.1% and enile vaginitis in 13%. The incidence of other benign onditions as low. The aetiology of postmenopausal genial bleeding discussed.

Postmenopausal genital bleeding is a symptom for which medical aid is usually sought, because it is well know that the cause may be malignant process. Kraatsold (1962) reviewed 26 157 cases of postmenopausal bleeding collected from the literature. The cause was malignant in 48.7%. Pacheco & Kempers (1968) found that the rate *of mal gnancy as the aetiology of such bleeding* aried greatly in different investigations, the range being from 5.4–76.0%.

I this study the term postmenopausal bleedg is used for blood-stained discharge from the female genital tract occurring after an inter al of at least one year from cessation of menstruation t the appropriate time of life.

MATERIAL

The records were reviewed for all patients seen for postmenopausal genital bleeding in 1960–1963. The series consisted of 1 085 omen with postmenopausal genital bleeding. They were at least 45 years old and the time lapse from the last menstruation was at least one year. The median age was 58.0 years, lower quartile 53.0 years, upper quartile 64.4 years, range 45–87 years. The median postmenopausal interval was 8.5 years, lower quartile 3.1 years, upper quartile 14.7 years, range 1–40 years.

RESULTS

The pathological diagnosis, median age and me dian postmenopausal interval are shown in Table I for 1 085 patients with genital bleeding. The bleeding was caused by a malignant process in 28%. Endometrial or cervical carcinoma was involved in the majority of these cases. The median age and median postmenopausal interval were clearly higher in this group than in the nonmalignant cases.

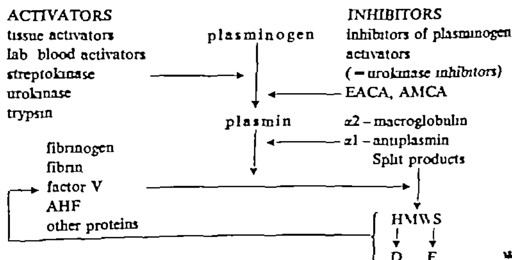
Atrophic endometrium was the most frequent cause of bleeding in the non-malignant group (20.5%). These patients were mostly older than the remainder and the postmenopausal interval was relatively long. In many of these cases senile vaginitis was also present. In 200 cases (18.5%) the bleeding was obviously due to a hormonal effect in the endometrium. The endometrial histology corresponded w th the proliferative phase of the fertile period in 57 cases, while 132 patients showed hyperplastic and 11 a clearly secretory endometrium. These patients were o erage younger than the remainder. This true in particular for the women showing secretory endometrium. I these the median ge wa 48.5 years and the median postmenopausal interval was 1.6 years. In 17.– the bleeding was due to an endometrial or cervical polyp. In 12.1% the cause was cervicitis with erosion

Gynaecologists

Menorrhagia may be caused by an increase in local fibrinolytic activity Cyklokapron reduces menorrhagic haemorrhages by an average of 50%

Women with average menstrual blood losses of over 80 ml have higher concentrations of plasminogen activators in the endometrium than those with lower blood losses. The resultant increase in local fibrinolytic activity is inhibited by Cyklokapron. The recommended dosage of Cyklokapron in menorrhagia is 1 g 3–6 times daily for 3–6 days. With a dosage of 3 g daily Nilsson and Rybo noted reductions in bleeding of 38 % compared with control cycles. With twice this dosage bleeding was reduced by 51 %. None of the 36 patients participating in the trial were obliged to discontinue treatment as a result of side-effects.

Reference: NILSSON L., RYBO G. Treatment of menorrhagia with an antifibrinolytic agent, tranexamic acid (AMCA). A double blind investigation. Acta Obstet Gynecol Scand. 46 (1967) p. 572.



metrial/cervical carcinoma in cases of postmenopausal bleeding is 1 2. In the present investigation the ratio is 1 1 The difference may be accounted for by the fact that the patients with cervical carcinoma in general are younger and as the result of more efficient information and improved diagnostics women today come under treatment at an earlier stage than previously On the other hand series have been published in which cervical carcinoma is markedly less frequent than endometrial carcinoma as a cause of postmenopausal bleeding (e.g. Woodruff et al., 1958).

The previously reported observation that the incidence of malignancy in postmenopausal bleeding rises with increasing age is corroborated by the present study

A non-malignant condition was involved in 72.. of the cases. The median age in these was clearly lower than in the malignant cases. Atrophic endometrium was diagnosed in 222 of these cases, or 20.5% which is in agreement with previous observations (Brewer & Miller 195.., Kraussold, 1962, Pacheco & Kempers, 1968). Of these patients 62 also showed uterine prolapse, which may have caused excoriation in the cervix leading to haemorrhage. Moreover senile vaginitis, which also is a potential cause of bleeding, is a frequent concomitant phenomenon. In the patients with atrophic endometrium the bleeding was scanty and these women were generally advanced in age.

The cases showing hormonal effect in the endometrium constitute an interesting group Such an effect was involved in 18.5% of the cases. An obvious oestrogen effect was observed in 17.5% (189 cases), which is slightly higher than in the report of Lehto & Kinnunen (1957) and Kraussold (196). These patients were on average younger than the remainder and the time lapse from the menopause was short. The bleeding was relatively abundant. Thirteen of these women had received oestrogen treatment prior to the postmenopausal bleeding. An increased use of oestrogen therapy lead to an increased incidence of postmenopausal endometrial bleeding due to an oestrogen effect It should be borne in mind, however that the cause of the bleeding may nonetheless be malignant process Hence a careful gynaecological examination in combination with vaginal smear and curettage is always necessary for a confident diagnosis. If no oestrogen has been given and the endometrium shows a clear oestrogen effect, the possible involvement of a pathological ovarian process should be borne in mind, but the possibility of adrenal oestrogen production should also be taken into account (Procopé 1968, 1970 *a*).

Eleven patients showed a clearly secretory endometrium and, consequently functioning corpus luteum. All of these patients belonged to the youngest age group in the series, their median age being 48.5 years. This permits the conclusion that an obvious activation of the ovaries was involved (Payne et al., 1959 Procopé, 1968, 1970 *b*).

The incidence of endometrial and cervical polyps in the present series was 17..%. This corresponds to previous findings (Kraussold, 1962)

In 12.1% of the cases cervicitis with erosion was involved. Half of these patients also showed uterine prolapse obviously contributing to the development of bleeding.

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Table I. *Pathological diagnosis no of cases, median age in years and median years after the menopause in 1 085 patients with postmenopausal bleeding*

	No. of cases	Per cent	Median age (y)	Median years after the menopause
Malignant conditions				
Endometrial carcinoma	154	14.2	61.5	10.5
Cervical carcinoma	141	13.0	59.3	9.8
Vaginal carcinoma	8	0.7	66.2	14.5
Vulva carcinoma	5	0.5	65.8	16.7
	308	28	60.9	10.5
Non malignant conditions				
Atrophic endometrium	[illegible]	20.5	58.6	9.6
Proliferative endometrium	57	5.3	54.5	3.4
Endometrial hyperplasia	13	[illegible]	53.6	[illegible]
Secretory endometrium	11	1.0	48.5	1.6
Endometrial polyp	91	8.4	57.1	7.0
Endometritis	11	1.0	54.5	6.8
Tuberculous endometritis	3	0.3	55.5	5.2
Submucous myoma	7	0.6	51.5	2.0
Cervicitis with erosion	131	[illegible]	[illegible]	9.3
Cervical polyp	95	8.8	58.3	9.4
Senile vaginitis	14	1.3	64.5	[illegible]
Vulval ulceration	1	0.1	(56.5)	(4)
Vulval condyloma	1	0.1	(48.0)	(1)
Urethral polyp	1	0.1	(63.0)	(15)
	777	72	56.8	6.8
Total	1 085	100	58.0	8.5

Senile vaginitis was involved in 1.3 %. These women were on average advanced in age. The incidence of other benign causes was very low.

The rate of malignant tumours in the various age groups of patients with postmenopausal genital bleeding is shown in Fig. 1. There is clearly a rise with increasing age.

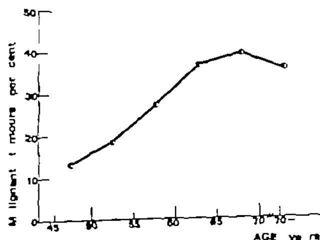


Fig. 1. Ratio of malignant tumours in the various age groups of patients with postmenopausal genital bleeding.

DISCUSSION

It is not easy to give an accurate definition of the postmenopausal state. One year without bleeding at the appropriate time of life is the criterion most generally applied. The term postmenopausal bleeding may be used when the time lapse from the menopause is at least one year (Brewer & Miller 1954, Sutherland & McBride, 1954, Lehto & Kinnunen, 1957, Klingenberg & Klausen, 1963, Novak 1964).

The great variation of the figures for a malignant aetiology in postmenopausal bleeding seen in different reports (Kraussold, 196[illegible], Pacheco & Kempers, 1968) may be attributed mainly to the inconsistent use of the terminology and the different character of the hospitals. In the present study the rate of malignancy was [illegible]8 %. This is a higher incidence than in the series of Lehto & Kinnunen (1957, 20.8 %) and Klingberg & Klausen (1963, 16.0 %) but lower than the mean value which Kraussold (196[illegible], 48.7 %) arrived at in his review of the literature. The most usual malignant cause is endometrial or cervical carcinoma. According to Kraussold (1967) the ratio of endo-

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RADIOGRAPHIC STUDY OF EXTRA AMNIOTICALLY INJECTED HYPERTONIC SALINE IN THERAPEUTIC ABORTION

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Abstract. Examination of radiographs from thirty-six cases after extra-amniotic injection of 20% NaCl containing contrast medium revealed as follows: (*a*) In most cases, the solution spread around the entire amniotic sac. (*b*) In no case was filling of the fallopian tubes observed. (*c*) In most cases, the solution disappeared from the uterus within four hours. (*d*) In some cases, the solution disappeared immediately from the uterus and appeared in the uterine veins and urinary bladder indicating intravascular injection. Effective myometrial contractions was obtained when the solution was retained in the uterus for one to two hours. Further retention did not shorten the interval between injection and delivery.

The mechanism by which an *intra-amniotic* injection of hypertonic saline induces abortion still challenges research (1). Also the mode of action of an *extra-amniotic* injection of hypertonic saline is still equally obscure. For example, it is not known whether the solution remains at the site where it is deposited or spreads around the amniotic sac. Nor is it known how soon the solution is absorbed by the decidual vessels. Another question is whether the interval between the injection and abortion varies with the time the solution is retained in the uterine cavity. The purpose of this paper is to clarify these points as an initial step in an investigation of the mode of action of an intra-uterine injection of hypertonic saline. An attempt was also made to find out whether the pain sometimes occurring in the iliac fossae after extra-amniotic injection of hypertonic saline is due to a passage of saline through the fallopian tubes into the abdominal cavity; whether the occasional leakage to the vagina can be counteracted by keeping the patients in bed after injection of the solution; and whether the tip of the catheter is apt to cause vascular damage resulting in intravascular injection of the solution.

MATERIAL AND METHODS

The material consisted of 36 healthy women in the 15th to 20th week of pregnancy with permission for therapeutic abortion.

Hypertonic saline was made radiographically visible by addition of contrast medium (*Isopaque Cerebral*, Nyco A S, Norway 15 ml per 100 ml of 20% saline). A Nelaton catheter No. 12 was passed through the cervix up between the uterine wall and the fetal membranes. The solution was injected in a volume varying with the length of pregnancy viz. 11.5 ml (equivalent to 10 ml of 20% saline) for each week of pregnancy. The first film was exposed after injection of 40 ml of the predetermined volume (test dose), a second one after injection of the rest of the dose, and then every hour for two to four hours. All radiographs were taken with the patient supine.

RESULTS

A. Intra-uterine spread of the saline-contrast medium

In 22 of the 36 cases the solution injected spread between the uterine wall and the membranes and surrounded the entire amniotic sac, either being evenly distributed (Fig. 1) or remaining mainly around the tip of the catheter with only a thin layer surrounding the amniotic sac (Fig. 2).

In eight cases the contrast medium spread only along one side of the uterus (Fig. 3) or only in the lower portion of the cavity (Fig. 4). The interval between the injection and abortion in these eight patients did not differ significantly

solution was retained, the patients were divided into two groups, viz. one in which the solution was no longer radiographically demonstrable after one hour (12 cases) and one where it was (20 cases). The interval between the injection and abortion proved shorter in the latter group (Fig. 5). Further retention, i.e. for all together two hours or more, did not result in still earlier abortion (Fig. 6). Two patients aborted after a relatively short interval (13 and 23 hours, respectively) in spite of the fact that the solution had disappeared from the uterus within one hour

D *Disappearance of the saline-contrast medium from the uterus*

In most cases the solution disappeared from the uterus within four hours (Fig 6). Theoretically the disappearance may be due to:

(*a*) Leakage to the vagina
(*b*) Absorption
( ) Intravascular injection

Leakage to the vagina. Leakage to the vagina was observed in 10 of the 12 cases in which the solution disappeared from the uterus within one hour (Fig. 1 *b*). To find out whether the frequency of leakage varied with posture, 14 patients were allowed to get up immediately after the injection, while 17 were kept in bed (Table I) No significant difference in frequency of leakage within one hour was found between the two



Fig 4 Radiograph after extra-amniotic injection of 170 ml saline-contrast medium. All of the solution can be seen in the lower part of the uterus.



Fig Radiograph after extra-amniotic injection of 220 ml saline-contrast medium. All the contrast medium can be seen along the right side of the uterus. Note the vertebral column of the fetus to the left.

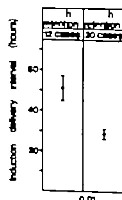


Fig Relation between the retention time of saline contrast medium and the interval between injection and abortion. Mean values and S. E. Wilcoxon rank-sum test were used for statistical analysis of the data.





Fig 1 Radiographs after extra-amniotic injection f 170 ml saline-contrast medium. ( ) Immediately after the injection. The solution has spread between the membranes and the uterine wall surrounding the whole amniotic sac. (*b*) One hour later The amount f intra-uterine solution is reduced due to leakage to the vagina.



Fig 2 Radiograph after extra-amniotic injection of 170 ml saline-contrast medium Most f the contrast medium is seen i th lower portio f the uterus, and only a thin layer surrounds the amniotic sac.

from that in patients in whom the solution spread around the entire amniotic sac.

The remaining six patients in whom the dose or part of it was accidentally injected intravascularly are discussed separately below

B. *Tubal filling*

No filling of the fallopian tubes was ever observed. This means that the pain sometimes occurring in the iliac fossa after extra-amniotic injection of hypertonic saline cannot be due to passage of saline through the fallopian tubes into the abdominal cavity

C *Relation between the retention time of the saline-contrast medium and the interval between injection and abortion*

In order to find out whether the interval between injection and abortion varied with the time the

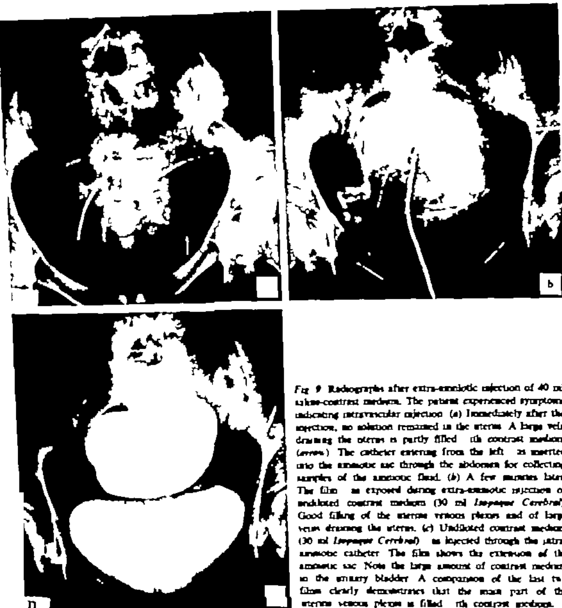


Fig. 9. Radiographs after extra-amniotic injection of 40 ml saline-contrast medium. The patient experienced symptoms indicating intravascular injection. (*a*) Immediately after the injection, no solution remained in the uterus. A large vein draining the uterus is partly filled with contrast medium (*arrow*). The catheter entering from the left was inserted into the amniotic sac through the abdomen for collecting samples of the amniotic fluid. (*b*) A few minutes later. The film was exposed during extra-amniotic injection of undiluted contrast medium (30 ml *Lipiodol Cerebral*). Good filling of the uterine venous plexus and of large veins draining the uterus. (*c*) Undiluted contrast medium (30 ml *Lipiodol Cerebral*) was injected through the intra-amniotic catheter. The film shows the extension of the amniotic sac. Note the large amount of contrast medium in the urinary bladder. A comparison of the last two films clearly demonstrates that the main part of the uterine venous plexus is filled with contrast medium.

patient no contrast medium was seen in the bladder at any time during the examination.

The six patients in whom the dose or part of it was accidentally injected intravascularly are discussed below.

Intravascular injection. In six patients the symptoms and the radiographic findings indicated intravascular injection. Immediately after the injection these patients experienced burning, flushing and tingling sensation as well as one or more of the following symptoms: headache, vertigo, nausea and vomiting. In four cases no contrast medium could be seen in the uterine cavity immediately after the injection (Fig. 9). In two of the cases, however, a thin layer of contrast medium was demonstrated in the uterus indicating that only part of the dose had been injected intravascularly. In all six patients contrast medium was demonstrated in fairly high concentration in the bladder within a few minutes of the injection.

In the four patients with complete intravascu-

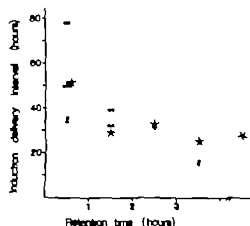


Fig 6. Relation between the retention time of the saline contrast medium and the interval between the injection and abortion. Since radiographs were exposed at hourly intervals, the points of retention (•) are shown midway between successive hours. ★ mean value. The table does not include the four patients in whom the entire dose was injected intravascularly or the four in whom the retention time was unknown since they were observed only for two hours and had by then demonstrable contrast medium in the uterus.

Table I *The effect of posture (in and out of bed) on vaginal leakage of saline-contrast medium*

	No of patients	Contrast medium in vagina one hour after injection
Out of bed	14	4
Confined to bed	17	8

Four patients in whom the entire dose had been injected intra ascularly nd one whose posture was uncertain, are excluded. The four-fold table test showed no statistically significant difference between the tw groups.

groups. In one of the patients kept in bed the radiographs showed periodic leakage probably due to uterine contractions.

Absorption Absorption by the decidua was estimated from the interval between the injection and the appearance of the contrast medium in the urinary bladder

In two patients contrast medium was seen in the bladder after one hour (Figs. 7 and 8) in eight patients, after two hours or more In 20



Fig 7 Radiograph after extra-amniotic injection of 195 ml saline-contrast medium. The film was exposed one hour after the injection. Almost all solution has disappeared from the uterus. Contrast medium can also be seen the urinary bladder Note the ertebral column f the fetus just abov the bladder



Fig 8 Radiograph afte tra-amniotic injectio of 195 ml saline contrast medium. The film, exposed on hour after the injection, showed small amount f contrast medium in the urinary bl dder nd the vagina. In the terus large mount f ntrast medium can b seen.

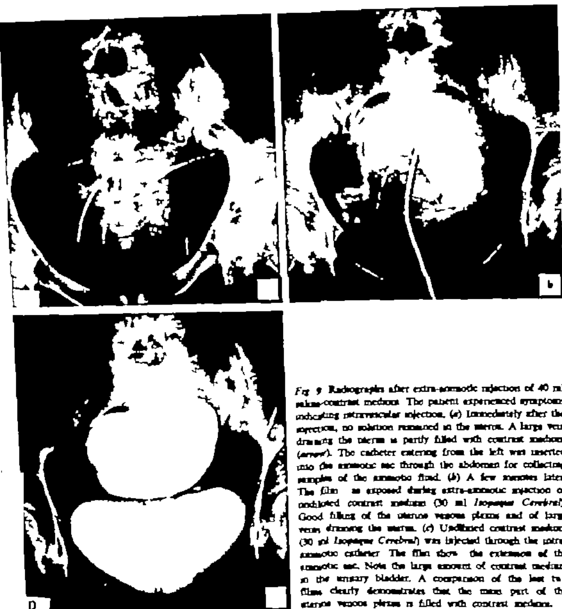


Fig. 9. Radiographs after extra-amniotic injection of 40 ml saline-contrast medium. The patient experienced symptoms indicating intravascular injection. (*a*) Immediately after the injection, no solution remained in the uterus. A large vein draining the uterus is partly filled with contrast medium (*arrow*). The catheter entering from the left was inserted into the amniotic sac through the abdomen for collecting samples of the amniotic fluid. (*b*) A few minutes later. The film was exposed during extra-amniotic injection of undiluted contrast medium (30 ml *Isopaque Cerebral*). Good filling of the uterine venous plexus and of large veins draining the uterus. (*c*) Undiluted contrast medium (30 ml *Isopaque Cerebral*) was injected through the intra-amniotic catheter. The film shows the extension of the amniotic sac. Note the large amount of contrast medium in the urinary bladder. A comparison of the last two films clearly demonstrates that the main part of the uterine venous plexus is filled with contrast medium.

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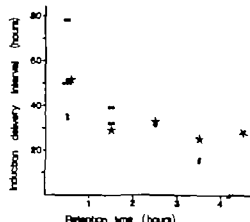


Fig 6 Relation between the retention time of the saline-contrast medium and the interval between the injection and abortion. Since radiographs were exposed at hourly intervals, the points of retention (•) are shown midway between successive hours. ★ mean value. The table does not include the four patients in whom the entire dose was injected intravascularly or the four in whom the retention time was unknown since they were observed only for two hours and had by then demonstrable contrast medium i the uterus.

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F g 7 Radiograph after extra-amniotic injection of 195 ml *saline-contrast medium. The film* was exposed one hour after the injection. Almost all solution has disappeared from th. uterus. Contrast medium can also be seen in the urinary bladder Note the vertebral column f the fetus just abov the bladder



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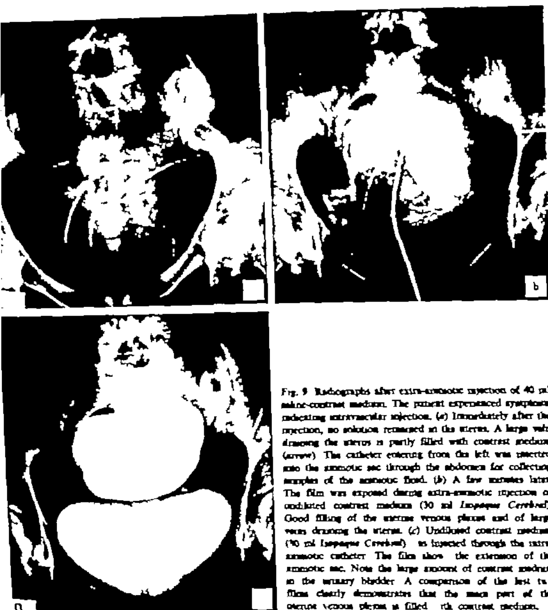


Fig. 9. Radiographs after extra-amniotic injection of 40 ml saline-contrast medium. The patient experienced symptoms indicating intravascular injection. (*a*) Immediately after the injection, no solution remained in the uterus. A large vein draining the uterus is partly filled with contrast medium (arrow). The catheter entering from the left was inserted into the amniotic sac through the abdomen for collecting samples of the amniotic fluid. (*b*) A few minutes later. The film was exposed during extra-amniotic injection of undiluted contrast medium (30 ml *Isopaque Cerebral*). Good filling of the uterine venous plexus and of large veins draining the uterus. (*c*) Undiluted contrast medium (90 ml Isopaque Cerebral) as injected through the intra-amniotic catheter. The film shows the extension of the amniotic sac. Note the large amount of contrast medium in the urinary bladder. A comparison of the last two films clearly demonstrates that the main part of the uterine venous plexus is filled with contrast medium.

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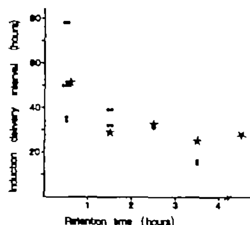


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THE INCIDENCE OF COMPLICATIONS FOLLOWING HYSTERECTOMY IN RELATION TO THE TIME INTERVAL BETWEEN CONE BIOPSY OF THE CERVIX AND HYSTERECTOMY

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Abstract. A series of 230 patients was studied with view to the effect of the time interval between cone biopsy of the cervix and hysterectomy on the postoperative febrile course and on the incidence of severe complications, such as abscess and/or haematoma in the true pelvis or in the abdominal wound. Fever as ell as severe complications were found to be most common in patients treated by hysterectomy 4–14 days after cone biopsy. The causes are discussed. It is concluded that cone biopsy and hysterectomy should be carried out at the same time, ith frozen section microscopy of the biopsy. Otherwise, the hysterectomy should be deferred until 5 or 6 weeks after the cone biopsy

T confirm or make a diagnosis of cervical carcinoma, stage 0 and at the same time to rule it an invasive carcinoma, cone biopsy of the cervix must be considered a necessary diagnostic procedure (2, 7 12, 13)

If it discloses cervical carcinoma stage 0 the recommended treatment is total hysterectomy with vaginal cuff" and in the case of stage 1 carcinoma, radical hysterectomy removing the uterus, the upper third of the vagina, and the parametria and, in menopausal or postmenopausal patients, both ovaries (6, 8, 11)

According to the literature there is a relationship between the incidence of complications and the time interval between the cone biopsy and the hysterectomy

In order to assess the postoperative course the present, as well as previous authors have investigated the incidence of pelvic complications (haematomas and/or abscesses) wound complications (haematomas and/or abscesses), and the occurrence of fever defined as an elevation of the temperature up to or over 38 0 C for 2 days after the hysterectomy not including the day of the operation. This definition is identical with the definition of "febrile morbidity" in previous publications.

For instance, Osoba (12) studied the postoperative course in 38 patients treated by hysterectomy 2 days to 8 weeks after cone biopsy Of the 38 patients 28 had the major operation 2–10 days after the cone biopsy and within this group 19 (64 3%) had febrile course. In the 10 remaining patients hysterectomy was carried out 12 days t 8 weeks after the cone biopsy within this group only one patient (10%) had a febrile course.

Cavanagh et al. (2) studying 66 patients, concluded that a time interval of less than 6 weeks between cone biopsy and hysterectomy resulted in an increased incidence of postoperative fever. They found a febrile morbidity of 44 6% among the patients who had operations within this interval, while among those with an interval exceeding 6 weeks they found a febrile morbidity of 30%

In a series of 27 patients Doran et al. (3) found febrile morbidity of 45% with a cone biopsy hysterectomy interval from 1 day to months. All patients who had the hysterectomy after 7–14 days developed postoperative fever

Malinak et al. (10) investigated the post

lar injection, the above mentioned symptoms appeared after injection of 40 ml of the solution. In an attempt to demonstrate the route by which the solution passed from the uterus to the general circulation, undiluted contrast medium was injected through the catheter immediately after the onset of the symptoms. Films exposed during this injection showed the presence of contrast medium in the uterine venous plexus and large veins draining the uterus (Fig. 9 *b*)

F *Fetuses*

All fetuses were dead and macerated when delivered.

DISCUSSION

Since the molecules of the contrast medium are much larger than the ions of the saline the contrast medium may have been absorbed by the decidua or transported to the amniotic fluid more slowly than the saline. On the other hand, the radiographic disappearance of the contrast medium may have been due partly to dilution. Examination of the radiographs therefore yield only indirect information about the retention and transport of the saline.

Effective myometrial contractions were obtained when the solution remained in the uterus for one to two hours. Further retention did not shorten the interval between injection and delivery. These findings suggest that the saline requires one to two hours to trigger off the mechanism that induces abortion several hours after the solution has left the uterus. But the present findings do not warrant any conclusion as to the possible nature of this mechanism.

The demonstrable presence of contrast medium in the bladder within one hour suggests that in these cases the solution had passed into the decidual vessels relatively rapidly. But only when the solution was injected intravascularly was the concentration in the blood high enough to produce symptoms.

ACKNOWLEDGEMENTS

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morbidity in patients whose hysterectomy was done more than 14 days after the cone biopsy presumably because the majority of the hysterectomies were carried out 18 days after the cone biopsy If the hysterectomy is performed either less than 2 days or more than 42 days after cone biopsy only 25% of the patients are reported to run a febrile postoperative course (13).

On the other hand, Kaufman et al. (5) found the length of the cone biopsy-hysterectomy time interval to be of no importance to the postoperative course, and Cavanagh et al. (2) found an equal number with fever during the first 6 weeks after the cone biopsy Only if the hysterectomy was delayed for more than 6 weeks after the cone biopsy did they find a lower febrile morbidity

But more important than the febrile morbidity are the severe complications, such as abscesses and haematomas in the true pelvis or in the abdominal wound In our material pelvic complications occurred only in patients with cone biopsy hysterectomy interval of 4–14 days, and during this interval too the wound complications reached a peak

This is in accurate keeping with Malinak et al. (10) who found pelvic complications, wound complications, as well as one death among patients who had hysterectomy 2–18 days after cone biopsy whereas no severe complications occurred among patients who had a later hysterectomy

The histological changes in the wound left on the cervix by the cone biopsy have been described mainly by Malinak et al. (10) and help to explain why the incidence of complications is highest during the first 3 weeks after the cone biopsy

From the 2nd until the 5th–7th day after cone biopsy increasing inflammatory changes are seen, at the outset in the form of fibrinous covering. Gradually increasing infiltration with inflammatory cells appears, and purulent exudate is seen on the wound surface These changes reach a maximum about the 10th day when the defect in the cervix looks like a purulent, infected wound. In this exudate there may be clusters of bacteria which occasionally infiltrate the underlying stroma In the course of the subsequent 10 days the changes gradually subside. Now the cervical wound is predominantly granulation tissue in which roundcell infiltration occurs and the infiltration with polymorphonuclear leucocytes decreases. About 4 weeks after the cone biopsy re-epithelization sets in, and as a rule it has been completed 6 weeks after the cone biopsy

Considering the results of the present study as well as of those previously reported in the literature, it must be recommended that hysterectomy be delayed until 3–4 weeks, preferably 5–6 weeks after cone biopsy (13).

Otherwise hysterectomy should be carried out within 48 hours of the cone biopsy but the best procedure appears to be cone biopsy and hysterectomy in the same stage (4 5 7 10)

This shortens the stay in hospital and spares the patient more than one anaesthetic. Moreover we avoid the common complications of cone biopsy especially secondary haemorrhage (1 9)

This procedure requires frozen-section examination of the cone biopsies. Where this is practised as a routine, satisfactory agreement between the frozen sections and paraffin sections has been reported (3 5). However Guerriero et al. (4) report a 16% rate of error but after sufficient experience and routine had been obtained, their material also showed satisfactory agreement between the frozen sections and paraffin sections. The time required to study the cone biopsy is reported to be 15–20 minutes, rarely up to 45 minutes.

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Table I *Pelvic complications*

	Total no. of patients	Patients with complications	%
< 3 days	21	0	—
4–14 days	161	22	14
> 15 days	48	0	—
Total	230	22	10

Table II *Wound complications*

	Total no of patients	Patients with complications	%
< 3 days	21		10
4–14 days	161	22	14
> 15 days	48	2	4
Total	230	26	11

operative course in 124 cases of hysterectomy following cone biopsy Out of 86 patients who had their hysterectomy 1–18 days after the cone biopsy 56 (65 1 %) had a febrile course, while out of 38 patients whose hysterectomies were 19–152 days after the cone biopsy 17 (44 7 %) had a febrile postoperative course.

Lastly Williams et al. (13) in a review of the literature, found a febrile morbidity of 25% if the hysterectomy was carried out within 2 days after the cone biopsy and of 66 % if the hysterectomy was done on the 4th–7th day following cone biopsy The longer the interval the lower the febrile morbidity At an interval exceeding 42 days only 25% were febrile

PRESENT INVESTIGATIONS MATERIAL AND METHODS

The series comprises 230 patients from the cancer prophylactic survey of Lolland-Falster 1967 1969 in whom cervical carcinoma, stage 0 or stage 1 a, was found by cone biopsy and who were treated by simple or extended hysterectomy

The treatment was performed in the three surgical departments of the Lolland-Falster hospitals, and from August 1968 also in the Department of Gynaecology and Obstetrics of the Central Hospital, Nykøbing Falster.

The cone biopsies were carried out with a cold-knife technique. All the specimens were studied in the same Department of Pathology by the same 3 pathologists. When the histological report was received (after 4 days) abdominal hysterectomy was carried out in a later stage. The time interval between cone biopsy and hysterectomy ranged from days to 2 months, but nearly all the hysterectomies were undertaken within 4 weeks of the cone biopsy

Table III *Postoperative febrile morbidity*

	Total no. of patient	Patients with complications	
< 3 days	21	8	38
4–14 days	161	102	64
> 15 days	48	24	50
Total	230	134	58

RESULTS

From Table I it is apparent that out of 230 patients 22 developed pelvic complications. All were in the group of hysterectomies done 4-14 days after the cone biopsy

Table II gives the distribution of the wound complications. A total of 26 out of the 230 patients developed haematoma or abscess in the abdominal wound. However the majority of the wound complications occured following hysterectomies carried out 4–14 days after the cone biopsy while only a few wound complications were observed in patients who had their hysterectomy after an interval exceeding 14 days.

134 out of 230 patients (58 %) had a febrile postoperative course. Table III shows that the febrile morbidity was highest after hysterectomy from 4–14 days after the cone biopsy

DISCUSSION

According to the literature a febrile postoperative course and severe complications are more common when hysterectomy is carried out at a later stage than cone biopsy ("delayed hysterectomy") (2, 3 7 10, 12 13)

The present study showed the highest incidence of febrile morbidity and severe post operative complications in patients treated by hysterectomy 4–14 days after the cone biopsy Following hysterectomy carried out 3 days and from 4–14 days after the cone biopsy the febrile morbidity was 38 % and 64 respectively This accords with previous findings (10 12, 13) Doran et al. (3) found a febrile course after all hysterectomies undertaken 7–14 days after cone biopsy On the other hand, they found a lower febrile

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ON INTRAUTERINE DEATH

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Abstract The material comprised 56 stillborn fetuses from the Central Hospital of Tampere, during the period from January 1 1968, to December 31, 1969 It was shown that maternal causes (27% of the total), and causes derived from the placenta (42% of the total), were the most significant findings in elucidating the causes of intrauterine deaths

Determination of the cause of intrauterine death often appears to be an insurmountable problem. In Finland alone several articles on this subject have been published (e.g. 16, 17 21). In the studies concerned with the causes of perinatal death generally attention has also been paid to intrauterine fetal death (1) However there are still relatively few references to the additional information yielded by the pathologic-anatomic examination of the placenta in this respect. Placental examination has been developed to supplement the often incomplete picture autopsy gives of the case (4 10 11) Further there are papers on clinical studies carried out e.g. in England (24) as well as other extensive articles on perinatal death (18, 19 20). On the basis of these publications several important maternal and fetal findings of significance in the intrauterine death mechanism have emerged.

T ble 1 *Stillbirths and perinatal mortality in the Central Hospital of Tampere 1968–1969*

	Live births	Still-births	Total	%	Perinatal mortality ( )
1968	4 308	32	4 346	0.74	1 6
1969	4 445	4	4 449	0 54	1 5
Total	8 753	56	9 335	0 60	1 6

MATERIAL AND METHODS

The subjects are all newborns, with zero Apgar score. Their birth weights were at least 600 g.

The material was collected between January 1 1968, and December 31, 1969 in the Central Hospital of Tampere and comprises both autopsy findings in stillborn fetuses and the records of their mothers. During the period of study 9 335 children were born, of whom 56 or 0.6% were stillborn.

In connection with routine autopsies samples for listeriosis and toxoplasmosis were taken in all cases from the brain, liver, lungs, and placenta. During the puerperium the mothers were also subjected to serological examinations for listeriosis, toxoplasmosis, and cytomegalo-antigens as well as routine blood and urine analyses and glucose tolerance test, chromosomal culture, and X-ray examination, subject to judgement

For the examination of the placenta used modified Becker method (2) in the gross examination, and in the microscopic examination the criteria defined by Kloos & Vogel (11)

The causes of intrauterine death were grouped with slight modifications according to Simpson (22) and Emmrich & Malter (4).

RESULTS

Thirty mothers (54%) of stillborn infants were primiparae. Only three of the 56 mothers were less than 20-years-old. Fortysix were in the age group 20 to 34 years, and the remaining seven mothers were over 35 Only one of the 56 mothers had not been to an antenatal clinic during her pregnancy In the examinations at either antenatal clinics or hospitals an obvious pregnancy defect or some other disease had been detected in 44 mothers.

More than half of the deliveries took place between the 33rd and 40th weeks of pregnancy The shortest pregnancy lasted 25 weeks and the

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Table III. *Autopsy findings in stillbirths*

	Main finding	Subsidiary finding	Remarks
1. *Pathological findings in mother*			
Late toxaemia	6	7	Widespread placental infarctions in 6
Anaemia	1	3	Widespread placental infarctions in 1
Diabetes mellitus	2	—	Embryonal persistent placenta in 2
Pyelonephritis	—	2	—
Vague fever	2	—	Two unclear cases
Toxic goitre	—	1	—
Gall bladder infection	—	1	—
Myomata	—	1	Placenta praevia
Hepatosis	3	1	Embryonal persistent placenta in 3
Cervical insufficiency	—	1	Shirodkar stitch
Hemorrhagic shock	1	—	Rupture of rectus abdominis muscle in multipara
Total	15		
2. *Combined findings in mother and infant*			
Prolonged gestation	2	1	*Deficiency of umbilical artery in 1* retarded placenta in 2
ABO-incompatibility	1	—	Embryonal persistent placenta
Rh-incompatibility	2	—	Embryonal persistent placenta in 2
Prior threatened abortion	4	—	Placental fibrosis in 2, placental persistence and leucocytosis in 1 ramification defects in 1
Total	9		
3. *Findings in infant*			
Malformations	4	1	1 acardius, 1 with multiple anomalies, 1 omphalocele and lack of umbilical artery 1 omphalic and cardiac anomaly and 1 anencephalus
Twins, monochorial	—	5	Arterio-venous shunt in 2
Twins, dichorial	—	2	—
Intrauterine infection of infant	4	—	Intrauterine pneumonia in 1 ruptured membranes for over 24 hrs in 2, sepsis in 1
Total	8		
4. *Findings in placenta and umbilical cord*			
Implantation deficiencies			
Velamentous insertion of umbilical cord	3	—	2 A-twins
Lack of umbilical artery	—	2	1 prolonged gestation, 1 case of anomalies
Placenta circumvallata	—	2	2 abruption of placenta
Placenta praevia	1	—	Uterus myomatosus
Placentation defects			
Embryonal permanence	—	12	2 diabetes mellitus, 2 hepatosis, 2 Rh-incompatibility 1 recent listeriosis, 1 ABO-incompatibility
Ramification defect	—	7	6 toxaemias, 1 listeriosis
Retardation	—	5	2 cases of prolonged gestation
Circulation defects			
Intervillous thrombosis	—	5	—
Numerous infarctions 40	5	7	1 anaemic mother 6 toxaemias, 1 anencephalus, 4 small-for-date infants
Fibrosis	—	6	2 listeriosis, 1 umbilical cord strangulation, 1 prolonged gestation, 2 with prior threatened abortion
Arterio-venous shunt	1	1	2 A-twins
Abruptio placentae	8	—	Rupture of peripheral sinus in 2
Rupture of peripheral sinus	—	3	—
Inflammatory reaction of placenta	—	5	1 recent listeriosis, intrauterine pneumonia in 1 ruptured membranes for over 24 hrs in 3
Umbilical cord defects			
Strangulation in umbilical cord	4	—	1 B-twin
Compression of umbilical cord	—	1	—
Cord prolapse	2	—	1 B-twin
Embryonal membrane defects			
Membranes ruptured for over 4 hours prior to birth	—	4	Amniotic fluid drained for 5 weeks in 1
Total	24		

longest 43 weeks. The newborns comprised 32 males and 24 females.

Before the onset of labour 42 infants (75%) were confirmed to be dead, and 14 infants died during labour (25%)

The clinical course of the deliveries is shown in Table II

The 14 intrapartum fetal deaths all resulted from severe asphyxia. In four cases the death was caused by toxaemia of pregnancy including a pathologic placenta, in three cases prolonged gestation as well as placental changes, in two cases a prolapse of the umbilical cord, in three cases intrauterine fetal infection, and in the remaining three cases death resulted from miscellaneous factors.

Table III presents cases according to the main findings found at autopsy Both antepartum and intrapartum deaths are detailed.

Table IV is a summary of the significance of various factors in the cause of intrauterine fetal deaths.

DISCUSSION

In the material under discussion the fetus was dead prior to arrival in the hospital in 60% of cases and died in the hospital in 40% of cases. The corresponding figures in Potter and Davis's report (18) were 50% Antepartum deaths totalled 75% and intrapartum deaths 25%

On the basis of this material toxaemia of pregnancy was the most common finding in fetal death (10 7%) resulting from "maternal causes Toxaemia has generally been found to be one of the most important of antepartum and intra partum causes of death (15 23 13) Besides maternal diabetes mellitus, hepatosis (9 26) was found to be a significant finding in fetal death (about 5%) *Typical maturing defects of placenta* mimicing the so-called "embryonal persistence were a new observation in the hepatosis cases.

In the present material among the combined *maternal* and fetal causes listeriosis played a significant role in 4 cases or 7% while in for example, a German series it was only 1% (4) In three of the present cases the infection seemed to be chronic. The fourth was a recent case and other findings included intrauterine pneumonia (12) No cases of toxoplasmosis cases were encountered although in Thieme & Klose's (23) material it was the most common cause of specific infections. In the two cases of Rh-incompatibility *placental findings were characteristic*, although in one of these, serologic findings were at times inconstant (re. maturing defects of the placenta)

Among the fetal causes there were five cases of malformation (about 10%) four of which we considered lethal. The fifth one was an anencephalic fetus with numerous infarctions in the *placenta in addition. In Saxen's (21)* Finnish material the proportion of malformations was 20% Intrauterine infection of the infant was found in four cases; two had, in addition to the *aforementioned listeriosis*, "*premature* rupture of the membranes for long time and one of them had sepsis, the origin of which remained open.

Among the "placental and umbilical cord" *factors implantation* defects (24) included three velamentous insertions of the umbilical cord, their role in the death of one of twins was considered to be significant in at least two cases. Total

Table II. *The clinical course of delivery of stillborn infants*

	Died before onset of labour (Total 42)	Died during labour (Total 14)
Dead prio t arrival in hospital	34	—
Died while in hospital	8	14
Spontaneous delivery	22	8
Induction of delivery with oxytocics	19	3
Caesarean section	1	3
Infant's weight 2 500 g or more	15	7
Infant's weight 1 250 g–2 499 g	18	6
Infant's weight 600 g–1 49 g	9	1
Delivery in the 23rd–33rd week of pregnancy	6	4
Delivery in the 34th–37th week of pregnancy	21	4
Delivery in the 38th–41st week of pregnancy	13	5
Delivery in the 42nd–43rd week of pregnancy	2	1
Labour lasted less than 12 hours		6
Labour lasted 12 24 hours		7
Labour lasted more than 24 hours		1
Normal occiput presentation		9
Frontal presentation		1
Footli g presentation		3
Breech presentation		1

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Table IV *Distribution of the principle causes of deaths in stillborn infants*

	Number	%
1 Maternal causes	15	26.7
2 Maternal and fetal causes	9	16.1
3 Fetal causes	8	15.5
4 Placental causes	4	41 7
Total	56	100 0

placenta praevia in a myomatous uterus was found to be the cause of death in one case.

The most obvious of maturing defects (e.g. 11) were microscopically discernible so-called "embryonal persistence" changes in two cases of diabetes mellitus (e.g. 8) in two cases Rh incompatibility (e.g. 7) and in one of ABO-incompatibility Similar changes were encountered in three cases of hepatosis in the literature there is no prior mention of this. Placental retardation was seen most clearly in two cases of prolonged gestation (4)

Extensive placental infarction was found in cases of toxaemia (e.g. 14) but infarctions were seen also in the "small for-date cases without toxaemia (e.g. 10) Typical microscopic ramification defects were found especially in connection with toxaemia placentas, as Frank (5) has described.

The arterio-venous shunt characterictic of transfusion syndrome described by Benirschke & Driscoll (3) was found in the amniotic blood vessels of the placenta in two twin placentas, and in at least one of them it seemed to be the cause of fetal death.

Premature separation of the placenta was common as a *main finding in intrauterine death* (8 cases or 14%) Lilien (13) and Gruenwald et al. (6) reported the same incidence

Among the umbilical cord defects both strangulation and prolapse seemed to have significance in the fetal death in a total of six cases (about 12%) which is of the same order of magnitude as in e.g. Theme & Klose s material (23) The significance of strangulation by the umbilical cord is commonly considered to be rather unimportant although e g. Lilien (13) has found the "tight nuchal cord" to be a significant finding in intra partum deaths.

Premature rupture of membranes was at least a partial finding in four fetal deaths, in two of which an intrauterine infection of the fetus was observed.

It is often difficult or even nearly impossible to decide which cases have been "unsolved" The proportion of unsolved cases is rather large especially in those series which do not include placental examinations, as e.g. Saxén 38% (21), but even when placental examination is included, the number of unclear cases often remains high, as in 14% of Theme & Klose s series (23).

In Table III the main findings represent the opinion of the pathologist as to the main causes in the mechanism for the fetal death. It has not always been possible for example, to diagnose asphyxia before the death and mainly in the cases of intrapartum fetal death it has been easier for the obstetrician to determine correct diagnosis.

The role of placental findings in elucidating intrauterine causes of death is important and in the present work is highly significant. In 42% *of cases a placental change was found to* be the cause of fetal death. Emmrich & Mälzer (4) have also observed significant placental findings in more than 50% of cases, when clarifying the causes of deaths of stillborn infants.

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PREMENSTRUAL SYNDROME AND PHYSICAL EXERCISE

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Abstract. The relationship between premenstrual syndrome and physical exercise was studied with the aid of questionnaires in series of 748 female university students. Girls ho practised sports showed less symptoms of central nervous tension, particularly headache. Menstrual dysmenorrhoea also was less frequent in the athletes than in the control group. On the other hand no correlation was observed between premenstrual dysmenorrhoea, oedema and the practice of sports. Gymnastics had practically no effect on the various premenstrual or menstrual symptoms. The favourable influence of sports is attributed mainly to an improved circulatory capacity

I this paper the term premenstrual syndrome is used for the recurrent somatic or psychic symptoms which may occur in relation to menstruation. The purpose of study was to analyse the effect of psysical exercise on the various components of this syndrome.

There is consensus of opinion that sports and gymnastics have favourable effect on the pre menstrual syndrome However the relationship between the various symptoms and different forms of physical exercise has not been closely analysed

MATERIAL AND METHODS

The series consisted of female students from the Helsinki University including the Institute of Physical Education, the Helsinki School of Economics and the Institute of Physical Education, Jyväskylä. The data was obtained by means of questionnaire containing 60 questions requiring an answer "yes or "no Of 1 000 questionnaires distributed 748 were returned Since the object of the study was to estimate the effect of physical exercise, the questionnaire as sent to all students of the Institutes of Physical Education in both Helsinki and Jyväskylä and only to every eight of the female students of other faculties.

The age distribution as as follow 20 years 15.3%, 1 22 years 39.3%, 23– 4 years 26%, 25–26 years 10 1 and 27 years 9.3%

It is quite obvious that the girls accepted by the Institutes of Physical Education constitute group with outstanding physical attributes. The proportion of unmarried girls was higher in this group than in the remainder of the series (88% against 79%), and late menarche was also more frequent (after 15 years in 12% against 6.8% of the students of other faculties). The results for the two groups of students of Physical Education were pooled. In addition, the replies were classified for analysis according to the presence or absence of an expressed interest in sports and gymnastics. The sports most commonly practised were cross-country track finding, skiing, swimming and athletics.

The symptoms of premenstrual syndrome ere divided into three groups. In the first group the main symptom was pain ("dysmenorrhoea"). However headache was considered as due to premenstrual tension. In the second group of symptoms the central nervous system was mainly involved ("premenstrual tension") The third group consisted of oedema and symptoms from various organs ("miscellaneous symptoms").

The severity of the syndrome was also estimated by the replies in regard to the use of analgesics and medical advice sought on account of the symptoms

Some symptoms obviously are vague. A reported pre menstrual eight increase, for example, cannot be accepted without reserve in the absence of evidence.

In the statistical analyses the chi-square test was used. I the tables the significance of the differences is indicated ith asterisks as follows: P 0.05 (almost significant) P 0.01 (significant) = ** $P < 0.001$ (highly significant) = **

RESULTS

The Institutes f Physical Education as compared with the other faculties

A total of 136 replies was obtained from students of Physical Education. As seen in Table I there were some differences between these and other students. In the Physical Education group low back pain was less frequent but, on the other hand, menstrual pelvic pain was sligthly more

Table III. *The frequency of premenstrual oedema and othe symptoms (%) in 136 young women studying physical education and 612 female students from other faculties*

	Oedema					Other symptoms		
Faculty	Swelling of abdomen	Weight increase	Changed frequency of micturition	Mastalgia	Swelling of breasts	Constipation	Diarrhoea	*N*
Faculties of Physical Education	57	32	14	42	43	11	20	136
Other faculties	49	30	21	47	47	14	18	612
Significance of difference	—	—	—	—	—	—	—	

Effect of competitive sports

The series comprised 41 competitors. On average, this group of girls had the same favourable figures for dysmenorrhoea as the general group of athletes. The low frequency of headache in the group of competing athletes is striking (5 and 6%, Table V): it is even lower than in the general group of athletes (15 and 12%).

The competing athletes had sought medical advice relatively often (in 24%). This may however be due to more effective care rather than to greater need of help (Table IV).

DISCUSSION

Frequency of symptoms. When subjective statements are the source of information on a group of symptoms, it is obvious that the frequencies in different series will vary widely. The wording of a question influences the reply. However, if care is taken to elicit all manifestations, even slight ones, it is noteworthy that the frequency of some symptoms approaches 100% (12). In our opinion this is quite natural. Certain types of premenstrual and menstrual pain and psychic disturbances are physiological. However, if the

T ble IV. *The frequency of dysmenorrhoea ( ) correlated ith the mode of physical exercise*

The symbols of statistical gnificance: see Material and methods

Mode of exercise	Premenstrual pelvic pain	Premenstrual pelvic pain, severe	Premenstrual low back pain	Premenstrual low back pain, severe	Menstrual pelvic pain	Menstrual pelvic pain, severe	Menstrual low back pain	Menstrual low back pain, severe	Analgesics	Bed rest	Visited doctor	N
1 Gymnastics	70	13	43	8	92	35	64	12	39	16	20	442
2 No gymnastics	64	12	40	3	92	37	65	12	43	18	20	294
Significance of difference	—	—	—	—	—	—	—	—	—	—	—	
Sports regularly	69	15	36	7	92	24	54	8	29	14	21	135
Others	66	15	44	7	91	39	69	14	43	17	20	533
Significance of difference	—	—		—	—		⏟	⏟		—	—	
3 All answering "Sports-yes" compared with group 4	—	—	—	—	—	—	⏞	⏞	—	—	—	363
Competitors	66	10	39	10	80	28	68	15	29	12	24	41
Significance of difference in comparison with group 3	—	—	—	—	—	—	—	—	—	—	—	

Table I *The frequency of dysmenorrhoea ( ) in 136 young women studying physical education and 612 female students from other faculties*

Statistical significance of difference is expressed by asterisks (see "Material and methods")

Faculty	Premenstrual pelvic pain	Premenstrual pelvic pain, severe	Premenstrual low back pain	Premenstrual low back pain, severe	Menstrual pelvic pain	Menstrual pelvic pain, severe	Menstrual low back pain	Menstrual low back pain, severe	Analgesics	Bed rest	Visited doct	N
Institute of Physical Education	63	14	33	8	92	66	46	11	30	15	22	136
Other faculties	66	13	45	7	75	55	54	13	43	17	20	612
Significance of difference	—	—		—						—	—	[illegible]

common. The need of analgesics was, however less frequent in the "Physical Education group"

Table II shows the frequency of symptoms of premenstrual tension. The overall frequency was lower in the Physical Education group than in the remainder The difference was most striking for headache, but it was also obvious for anxiety and depression.

As regards "miscellaneous symptoms" no clear differences between the two groups were observed (Table III).

Effect of sports and gymnastics on the premenstrual syndrome

A group of girls who had a regular habit of gymnastics was compared with a group of athletes. Four competing gymnasts and 37 competing athletes were considered as a separate group.

The results are shown in Tables IV V and VI. The gymnasts did not differ favourably from the control group in regard to either pain or any other symptoms. The slight differences that occurred were rather to the disadvantage of the gymnasts. *Premenstrual restlessness*, abdominal swelling and weight increase, for example, were more frequent in this group (Tables V and VI). On the other hand athletes compared favourably with the control group particularly in regard to pain and to tension. However it is noteworthy that premenstrual pain was not influenced by physical exercise *in any form* whilst menstrual pain and headache were less frequent among the athletes than among the remainder of the series (Table IV) The favourable figures for the students of Physical Education (Tables I and II) thus seem to be due more to the practice of sports than gymnastics.

Table II *The frequency of premenstrual tension (%) in 136 young women studying physical education and 61 female students from other faculties*

Statistical significance of difference is expressed by asterisks (see "Material and methods")

Faculty	Premenstrual headache	Menstrual headache	Premenstrual nervousness	Irritability	Anxiety	Depression and fatigue	Premenstrual nausea	Menstrual nausea	Vomiting	N
Institute of Physical Education	11 4	10.4	50 0	57.5	5 7	30.0	6	16	11	136
Other faculties	26 8	1 6	60.8	70 0	16.5	43.[illegible]	11	18	9	612
Significance of difference							—	—	—	

Table VI. *Frequency of oedema and other symptoms" correlated with the mode of physical exercise*

Note the tendency to higher frequency of oedema in the group of women preferring gymnastics. Sport does not correlate with oedema. Significance of difference is expressed by asterisks (see Material and methods")

Mode of exercise	Oedema: Swelling of abdomen	Oedema: Weight increase	Oedema: Changed frequency of micturition	Oedema: Mastalgia	Oedema: Swelling of breasts	Other symptoms: Rectal pressure sensation	Other symptoms: Constipation	Other symptoms: Diarrhoea	*N*
1 Gymnastics	53	34	20	43	49	18	11	18	442
2 No gymnastics	45	25	18	40	49	18	16	18	294
Significance of difference			—	—	—	—	—	—	
3 Sports regularly	53	31	18	43	46	11	12	21	155
4 Others	50	31	20	42	48	19	13	17	533
Significance of difference	—	—	—	—	—		—	—	
5 All answering "Sport-yes compared with group 4	—	—	—	—	—	—	—	—	363
6 Competitors	44	29	15	40	37	10	12	22	41
Statistical difference between groups 3 and 6	—	—	—	—	—	—	—	—	

peripheral skin temperature by -7 C. All these changes were prevented by the administration of reserpine.

Premenstrual pelvic pain seems thus to be closely connected with a stimulation of a-receptors in the myometrium. Since sports had no effect on premenstrual pain, the mechanism of the favourable effect on the other symptoms of premenstrual syndrome can hardly be located to the autonomic nervous system.

A causal relationship has often been assumed between the symptoms of tension and oedema (1 4 15), but the recorded weight increases have been very slight (3). A premenstrual change in the distribution of fluid between different organs has therefore been suggested (15).

Since positive correlation has been observed between the symptoms of oedema and of tension (3 14), sports might be thought to reduce oedema of the central nervous system by accelerating the electrolyte metabolism. However in the present study no favourable effect of sports on the general symptoms of oedema was observed. Hence, another explanation must be sought. Oedema obviously is one cause of premenstrual tension, but there must be another, very strong aetiological factor which is favourably influenced by sports.

The tendency towards unsocial behaviour the accident-proneness (9), the increase in psychiatric hospital admission rate (8), the increase in suicide tendency (7) and the lowered test scores of female college students noted premenstrually (2) speak in favour of an accumulation of factors with detrimental effect on brain activity It is obvious that premenstrual tension can be explained as state of mental stress. A release of catecholamine occurs in the initial phase of stress. This leads to vasoconstriction causing increased circulatory resistance in the brain, with headache resulting. The vasoconstriction seems to be followed by vasodilatation (release of 5-hydroxytryptamine) only in women who have tendency towards menstrual migraine (11).

Hence premenstrual headache may be considered as secondary to the other symptoms of the

Table V *The frequency of premenstrual tension (%) correlated with the mode of physical exercise*
Statistical significance of difference is expressed by asterisks (see "Material and methods")

Mode of exercise	Premenstrual headache	Menstrual headache	Premenstrual nervousness	Premenstrual irritability	Premenstrual restlessness	Premenstrual anxiety	Premenstrual depression	Premenstrual nausea	Menstrual nausea	Vomiting	N
1 Gymnastics	22	21	58	68	45	14	41	8	15	2	442
2. No gymnastics	26	19	60	67	37	16	41	13	21	11	294
Significance of difference	—	—	—	—		—	—	—	—	—	
3 Sports regularly	15	12	47	61	36	7	31	7	13	8	155
4 Others	28	22	61	67	47	17	44	11	18	9	533
Significance of difference								—	—	—	
5. All answering "Sport yes compared with group 4					—	—			—	—	363
6. Competitors compared with group 3	5	6	50	50	32	6	28	10	13	5	41
			—		—	—	—	—	—	—	

frequency of these symptoms is estimated by the disability they cause as shown by the visits to doctors or the use of drugs, much lower figures are obtained. About one-fifth of the present series sought medical aid on account of premenstrual syndrome.

Effect of sports and gymnastics The slight effect of gymnastics, which even contrasted with the effect of sports for some symptoms, was a surprising finding. On the other hand sports had a strikingly favourable effect. This was particularly true for the various symptoms of premenstrual tension. The difference was most obvious for headache The occurrence of this symptom correlated negatively with the practice of sports.

Swelling and related symptoms showed practically no correlation with physical exercise. This is an interesting observation, because it has been suggested that the symptoms of tension are aetiologically directly related to sodium retention and oedema of the central nervous system (4).

A feeling of rectal pressure which had the lowest frequency in the group of athletes, did not seem to be connected with oedema it seemed to be associated with a tendency towards constipation, since the frequency of both these symptoms decreased with increased physical exercise.

Symptoms of dysmenorrhoea. These symptoms were less frequent among the athletes than among the gymnasts. However the difference was not so obvious as for premenstrual tension. It is striking that the frequency of premenstrual pelvic pain was not in the least influenced by the practice of sports. By contrast, menstrual pelvic and low-back pain were somewhat less common in the athletes. Ingman (6) reported a favourable effect of sports in 14 cases of dysmenorrhoea in a series of 107 athletes. On the other hand the regularity of the menstrual cycle and the amount of the menstrual flow were unfavourably influenced

In this connection it may be mentioned that cigarette smoking was also studied in the girls of the present series (14) Smoking showed a strong positive correlation with premenstrual pelvic pain but had practically no effect on menstrual pelvic pain and headache In general, the smoking habits of these female university students were relatively light the majority of smokers used less than 20 cigarettes a day It should be borne in mind however that even one cigarette means a clear ganglionic stimulation. Iisalo et al. (5), for instance showed that one cigarette was sufficient to raise the systolic pressure by 10–25 mmHg and to cause an acceleration of the pulse rate by 5–20/min and a drop of the

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premenstrual syndrome. The present results, however seem to indicate that the practice of sports does not only prevent headache, it has a prophylactic effect on the whole group of tension symptoms including the psychic components. Accepting the hypothesis that this effect is due to an improved—or perhaps better expressed, a stabilized—circulation in the brain, a logical connection can be discerned between the phenomena observed in this study The practice of sports increases the total capacity of the circulation and thus also the oxygenating capacity of the "cardiac-pulmonary pump"

It is a well-known fact that the circulation of the carotid region is relatively independent and differs from the circulation in general as regards regulation (cf for instance the so-called spare circulatory system in foetal intrauterine asphyxia) For this reason an increase of the circulatory capacity or in other words of the oxygenating capacity benefits precisely the brain most directly Premenstrually a greater circulatory capacity is required for satisfactory perfusion of the brain owing to the presence of some peripheral factor (oedema?) and the practice of sports obviously provides this additional capacity Thanks to the increased supply of oxygenated blood from the pulmonary circulation the regulatory mechanism of the cerebral circulation is able to cope with an increased peripheral resistance

The clear difference between gymnastics and the practice of sports in regard to the frequency of tension symptoms may be attributed to the fact that the effort involved in gymnastics is of *short duration. Therefore* it only results in a distribution of the blood flow which is detrimental to the brain while it benefits the skeletal muscles. For the same reason and because activation of the lungs is comparatively slight in connection with gymnastics, this form of physical exercise obviously does not lead to any noteworthy persistent increase in the capacity of the pulmonary circulation

The difference between sports and gymnastics was not so clear for menstrual pelvic pain as for the symptoms of tension. Menstrual pain obviously is only in part dependent on the autonomic nervous system and the circulatory capacity This is shown by the very weak correlation between these symptoms and the practice of sports or the smoking habit. In addition, individual local factors (dyscoordination between cervical dilatation and endometrial desquamation, the peritoneal spill of menstrual blood (10) cervical obstruction a lowered threshold for pain (13), etc) probably play a more important part in the aetiology of menstrual pain.

It seems obvious that the need for physical exercise is not sufficiently satisfied in modern society Facilities and guidance ought to be available explicitly for mass sports. Evidently mankind has strayed too far from a natural manner of living to find the way back without guidance.

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PROGESTERONE LEVELS IN AMNIOTIC FLUID AND PLASMA FROM WOMEN

I. *Levels during Normal Pregnancy*

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Abstract. Amniotic fluid samples from 45 early and 46 late pregnancies have been assayed for progesterone using a protein binding technique. Only 100 μl of amniotic fluid was needed for the determination. Simultaneous samples of plasma or serum have also been assayed. The highest progesterone levels in amniotic fluid were found between the 13th and 16th week of pregnancy when the levels were higher than in plasma. After this time there was a steady decline in amniotic fluid levels throughout pregnancy. The mean level during the 13th–16th week period was 55.3 ng/ml amniotic fluid and 26.4 ng/ml between the 37th and 40th week. In contrast plasma levels of progesterone increased with advancing pregnancy and exceeded amniotic fluid levels from the 21st–24th week onwards. The decrease in amniotic fluid progesterone concentration may be due to a relative decrease in placental progesterone synthesis and an increasing resistance to the passage of progesterone into the amniotic cavity.

As an approach to the study of fetal well-being the changes in the hormonal content of amniotic fluid have provoked considerable interest in the last few years. The present investigation is a part of a larger study of the difference between the amniotic fluid from women with complicated pregnancies and from normal obstetrical cases. As previous investigations of the normal levels of progesterone in amniotic fluid have involved only small numbers (11, 20, 23, 24), the present study was undertaken to study the levels in a large number of normal women. A prerequisite for this study was a rapid method for progesterone determinations in amniotic fluid.

MATERIAL AND METHODS

Subjects and samples

Transabdominal amniocentesis was performed on 91 women with normal pregnancies. The samples from the early part of pregnancy came from women ($n = 45$) admitted to the hospital for therapeutic abortion. The samples from the latter part of pregnancy came either from women ($n = 46$) of blood group Rh(−) with Rh isoimmunization who later gave birth to healthy babies of blood group Rh(−) or were obtained at elective Caesarean section in healthy women. None of the women was in labour when the samples were taken. A blood sample from an antecubital vein was taken at the same time as the amniocentesis.

A 0.8 × 80 mm needle was used for the puncture of the amniotic sac. Samples containing visible blood were discarded. The samples were centrifuged, filtered and stored at −15°C until analysed.

Materials

Corticosterone-1,2-^{3}H (specific activity 50.0 Ci/mmole), progesterone-1,2-^{3}H (specific activity 33.5 Ci/mmole), and 17α-hydroxyprogesterone-1,2-^{3}H (specific activity 49.2 Ci/mmole) were obtained from New England Nuclear Corporation.

Progesterone and 17α-hydroxyprogesterone were generously supplied by Schering AG, Germany. All other steroids were purchased from Ikapharm, Israel. All steroids have been tested for purity by thin layer chromatography or by separation on Sephadex LH-20 columns.

Light petroleum, Mallinckrodt 4980, lot TPY with boiling range of 30–60°C was used.

Florisil, 60–100 mesh (The Floridin Company Tallahassee, Florida, USA) was washed five times with glass distilled water and the finer particles were decanted. The remaining particles were dried at 120°C overnight. Only batches of Florisil that absorbed more than 80% of labelled steroids added to water were used in the assay.

Unless otherwise stated, all solvents were of analytical grade and were not purified further before use.

Precoated thin layer sheets (Silicagel with fluorescent indicator, Eastman chromagram sheet 6060 series number 1262) were washed twice by ascending chromatography in methanol before use.

Plasma containing the binding protein was obtained from healthy young women on contraceptive tablets con-

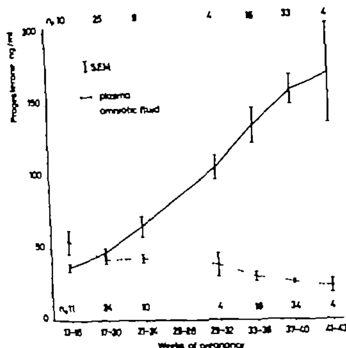


Fig. 2. Levels of progesterone in plasma and amniotic fluids from the same patients. The values are grouped in four weeks periods. , number of plasma samples in each four-week period, number of amniotic fluid samples in each four-week period.

plasma levels of progesterone rose steadily up to the 33rd week. The small increase of the mean levels seen from then up to the 41st to 43rd week period was not found to be significant. The progesterone levels in the amniotic fluid exhibited an opposite pattern. The highest levels were found between the 13th and 16th week of pregnancy followed by a gradual decline. The mean levels decreased from 55.3 ng per ml in the 13th–16th week period to 6.4 ng per ml between the 37th and 40th week. The difference between the 13th–16th week period and the 17th–20th week period was significant (0.05 $> p >$ 0.025). The difference between the 13th–16th eek period and the 33th–36th week period was also significant (0.005 $> p >$ 0.001) as was the difference between the 13th–16th week period and the 37th–40th week period (0.001 $> p$).

Between the 13th and 16th weeks the mean progesterone values were higher in amniotic fluid than in plasma but the difference was not significant. During the next 4-week period progesterone concentration was bout the same in both plasma and amniotic fluid. Between the 1st and 4th weeks the ratio of the progesterone concentration in plasma to the concentration in amniotic fluid was 1.5 1 from the 29th to the 32nd week 2.8 1 from the 33rd to the 36th week 4.4 1 and from the 37th to the 40th week 6.0 1

The correlation between progesterone concentration in amniotic fluid and corresponding values in plasma was found to be rather poor the correlation coefficient for the 13th–16th week being −0.02, for the 17th–20th week 0.30, 21st–24th week 0.74 and for the 37th–40th week −0.058. Progesterone concentrations in amniotic fluid were also found to have no significant correlation with total protein content of amniotic fluid as determined by modified biuret method (4), correlation coefficients being 0.26, 0.36 0.13 and 0.38 for the above mentioned periods.

No data was available regarding fetal or placental size for the early pregnancies but between the 37th and 40th weeks there were 23 deliveries within 7 days of sampling and the correlation coefficient between amniotic fluid progesterone levels and placental weight was found to be 0.505 which was significant at the 0.05 level. Correlation coefficients between progesterone in amniotic fluid and birthweight and also

Table I. *Percentage recovery of tritium labelled steroids (< 2 ng) added to amniotic fluid and extracted once with 20 volumes of petroleum ether (Mallinckrodt 4980 lot TPY)*

Steroid	Percentage recovery		n
Progesterone-1,2 ^{3}H	91 4	2.9	10
17α-hydroxyprogesterone 1,2^3 H	28.3	1.9	6
Corticosterone 1,2 ^{3}H	1 1	0.2	6

taining oestrogens (100 μg of mestranol or 100 μg of ethinyl oestradiol). The plasma was stored in one ml aliquots at −15°C.

Method

Samples of amniotic fluid (100 μl) were transferred into 3 ml extraction tubes equipped with well fitted glass stoppers. Petroleum ether (2 ml) was added, and the tubes were shaken by hand for one min. The petroleum ether was withdrawn into culture tubes using Pasteur pipettes and evaporated to dryness. Around 90% of the progesterone was extracted using this procedure.

The measurement was made by a competitive protein binding technique that has previously been described in detail (14 15) The unknown samples were read off a standard curve and corrected for recovery losses. No estimation of amniotic fluid blank was possible.

Sensitivity

The detection limit for progesterone in the competitive protein binding system used was 0.1 ng (14). As 0.1 ml of amniotic fluid was extracted with a recovery of 90% progesterone 1.1 ng per ml could be detected. All samples analysed were well above this level.

Specificity

The specificity of the described method for progesterone depends on the ability of the petroleum ether to exclude steroids that interfere in the protein binding system. As shown in Table I, a small portion of 17α-hydroxyprogesterone and corticosterone was extracted along with progesterone. These two steroids bind better in the protein system than progesterone (14) However their influence on the levels measured should be decreased by the low levels of these steroids present in amniotic fluid as compared with plasma. To evaluate the influence of these and other steroids on the levels measured after petroleum ether extraction only 11 samples were selected to represent all levels found and further purified on thin layer sheets (18). The mean level found after purification was 4_.8 ng per ml compared with 47.1 ng per ml after petroleum ether extraction only The difference was not statistically significant. In 8 of 11 samples the highest levels were found after purification on thin layer sheets. Therefore the interference from other steroids in the measurements of progesterone does not seem to be larger than in plasma samples during pregnancy using the same method (15).

Precision and accuracy

The precision was calculated from duplicate samples according to the method of Snedecor (22). In the range of 15–35 ng per ml the coefficient of variation was 8.4 (n = 36), between 36–55 ng per ml it was 11.0 (n 39) and above 55 ng per ml the coefficient of variation was 17.3% (n = 11).

As no progesterone free amniotic fluid was available, recovery studies were made from a pool containing 77.. ± 3 7 (s) ng per ml of progesterone. Known amounts of crystalline progesterone in ethanol were added to the pool and measured in the method. The accuracy of estimating added amounts of progesterone is shown in Fig. 1. As the extraction was carried out with 100 μl of amniotic fluid the addition of 4 ng of progesterone to the pool would be equal to amniotic fluid samples containing around 70 ng per ml. The levels recovered were in good agreement with the values expected. At the higher range of measurement the precision was poor

RESULTS

All progesterone levels reported here for both amniotic fluid and peripheral plasma were obtained after one petroleum ether extraction followed by competitive protein binding. Samples of amniotic fluid and blood were obtained from the 13th up to the 43rd week of pregnancy The results were grouped into 4-week periods. The levels found and the number of observations during each period are shown in Fig. 2. The mean

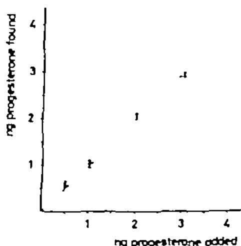


Fig 1 Accuracy of estimating progesterone added to a pool of amniotic fluid. The progesterone concentration in the pool was 77.. ± 3.6 ( ) per ml. The progesterone level of the pool was subtracted and correction made for losses in the extraction step. The *interrupted line* represents 100% recovery

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between progesterone and total protein in amniotic fluid were 0 123 and 0.381 neither of which was significant.

In 10 cases samples of cord blood plasma were obtained but in 8 of them the progesterone value was found to exceed 450 ng per ml which was the practical upper limit of the method used.

DISCUSSION

The plasma levels of progesterone during pregnancy found in this study agree very well with the values found in a larger group of women using the same method of progesterone determination (15) For amniotic fluid only a few determinations have been reported previously The levels found in late pregnancy are in accordance with those found by Schindler et al. (20) Somewhat higher mean levels during late pregnancy have been found by other investigators (11 23 24)

The most striking result of the present investigation was the high concentration in amniotic fluid found during the early part of pregnancy This pattern is in accordance with the findings of Zander and von Münstermann (24)

It is generally accepted that the placenta is the principal source of progesterone after the first trimester (6 15) As yet, very little is known about the routes by which progesterone is transferred to the amniotic fluid. It is evident that the formation and composition of amniotic fluid is not the same throughout pregnancy In early pregnancy amniotic fluid rather closely resembles maternal plasma with regard to its osmolality and content of electrolytes (10 11 16, 21) As pregnancy advances amniotic fluid becomes increasingly hypotonic and differs more and more from maternal plasma. Amniotic fluid in early pregnancy may be regarded as an ultrafiltrate of plasma but in late pregnancy the fluid formation is evidently more selective. The turnover rate for water is very rapid and amounts to about 500 ml per hour in late pregnancy (9 12, 13) In early pregnancy the relative turnover rate for water is still faster (9) Fetal swallowing in late pregnancy amounts to about 500 ml per day (19) and the input from fetal micturition is estimated to account for about 3–5% of liquor volume production at term (? 3) Thus, the greater part of water transfer and perhaps also of electrolytes must go by other routes. For other substances the turnover rates are largely unknown as are the relative importance of different transfer sites for any given substance. In early pregnancy the meshlike fetal skin may be an important site of entry (16) The volume of fluid in early pregnancy is closely related to fetal weight (2, 16)

The passage of any substance into the amniotic fluid depends not only upon molecular size but also upon its solubility in lipids and the degree of protein binding. For instance, a high degree of protein binding has been found to hamper the penetration of penicillin into the amniotic cavity (5) The decreasing levels of progesterone in amniotic fluid as pregnancy progresses may thus depend on increasing resistance to penetration.

The progesterone concentration in placental tissue may be of importance in explaining the difference between various stages of pregnancy The placental weight at 18–20 weeks is around 140 g (1) and at term 600–800 g, or a 5-fold increase. However the progesterone concentration per g of placental tissue is decreased (24). The corresponding progesterone concentrations in maternal plasma in this study were found to be around 47 and 160 ng per ml respectively which supports the view that the placental production of progesterone per unit weight is greater in early pregnancy Passage through the fetus is also a possible route as fetal blood contains high concentrations of progesterone. The levels of progesterone were above the measurable range of the method used even when 25 μl of cord blood plasma was extracted e.g. levels above 450 ng per ml

Changes in the amniotic fluid volume may account for some of the difference in amniotic fluid progesterone levels between early and late pregnancy The mean volume at 18 weeks is about ·80 ml (2, 8 21) and at 38 weeks around 1 000 ml (7) The total amount of progesterone present in the amniotic sac at 18 weeks is then about 1 160 ng and at 38 weeks about 2 600 ng. However it appears unlikely that changes in amniotic fluid volume would influence the concentration of progesterone to any great extent as no rise in progesterone concentrations was observed during the last few weeks of pregnancy when the volume of liquor is considered to decrease rather sharply from the peak volume

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PROGESTERONE LEVELS IN AMNIOTIC FLUID AND PLASMA FROM WOMEN

II. *Levels during Pregnancies Complicated by Rh-Immunization or Hepatosis Gravidarum*

L.-E. Jonasson and E. D. B. Johansson

From the Department of Obstetrics and Gynaecology (Head: Prof. Carl Gemzell), University Hospital, Uppsala, Sweden

Abstract. Using a protein binding technique, levels of progesterone were determined in 195 samples of amniotic fluid collected from 114 cases. Most of the pregnancies were complicated by Rh-immunization and parallel estimations of progesterone were performed in plasma or serum. The results show small variations between the various clinical groups. There was a significant rise in amniotic fluid progesterone concentration in pregnancies with very severe fetal erythroblastosis. Patients with hepatosis gravidarum showed a tendency towards high values both in amniotic fluid and in plasma. Progesterone determinations in amniotic fluid could be of limited practical value in the management of pregnancies complicated by Rh-immunization.

After the twelfth gestational week current opinion considers progesterone to be almost exclusively produced by the placenta. It might, therefore, be of interest to study the levels of progesterone in amniotic fluid as an indicator of placental function and to compare levels in amniotic fluid with those in peripheral plasma. Information about progesterone concentrations in amniotic fluid is limited, previous series being small and consisting mainly of samples from normal pregnancies (13, 18, 19).

The development of the protein binding technique for steroid estimation (10) has made it possible to examine large numbers of plasma samples. This technique has been applied to the estimation of progesterone in amniotic fluid in a large number of normal pregnancies (6). In the present study the progesterone levels in amniotic fluid were measured in pregnancies complicated by Rh-immunization or hepatosis gravidarum and related to the levels found during normal pregnancies.

MATERIAL AND METHODS

A total of 176 samples of amniotic fluid from 95 patients with Rh-immunization, 11 samples from 11 patients with hepatosis gravidarum and 8 samples from 8 patients with other complications of pregnancy were collected. In most cases samples of maternal venous blood were taken at the same time as the amniotic fluid samples.

The Rh pregnancies were grouped according to the clinical status of the infant. Group I represents those with mild disease, i.e. cord haemoglobin > 12.1 g/100 ml and requiring, at most, one exchange transfusion. Group II contains cases with moderate disease, cord haemoglobin within the range 8.1–12.0 g/100 ml or requiring 2–3 exchange transfusions. Group III consists of cases with severe disease, cord haemoglobin 8.0 g or lower or requiring more than 3 exchange transfusions. Group IV are cases where the pregnancy ended in intrauterine or postnatal fetal death. All the Rh-negative women had Rh positive children with a direct Coomb's test positive on the cord blood. In cases where intrauterine death from erythroblastosis occurred fetal blood grouping was not performed.

All cases classified as hepatosis gravidarum had the typical clinical symptoms of itching and sometimes slight icterus. All had abnormal liver function tests. None of them had any kind of iso-immunization and the pregnancies were otherwise normal. The diagnosis, hepatosis gravidarum, used here is synonymous with benign cholestasis of pregnancy (3, 4, 15).

All fluid samples were taken by abdominal amniocentesis. Most of the sampling was part of the routine management of Rh-immunized pregnancies. A few samples were taken during elective caesarean section before opening the uterine cavity. None of the patients were in labour. N

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MATERIAL AND METHODS

A total of 176 samples of amniotic fluid from 95 patients with Rh-immunization, 11 samples from 11 patients with hepatosis gravidarum and 8 samples from 8 patients with other complications of pregnancy were collected. In most cases samples of maternal venous blood were taken at the same time as the amniotic fluid samples.

The Rh pregnancies were grouped according to the clinical status of the infant. Group I represents those with mild disease, cord haemoglobin >12.1 g/100 ml and requiring, at most, one exchange transfusion. Group II contains cases with moderate disease, cord haemoglobin within the range 8.1–12.0 g/100 ml or requiring 2–3 exchange transfusions. Group III consists of cases with severe disease, cord haemoglobin 8.0 g or lower or requiring more than 3 exchange transfusions. Group IV are cases where the pregnancy ended in intrauterine or postnatal fetal death. All the Rh-negative women had Rh positive children with direct Coombs test positive on the cord blood. In cases where intrauterine death from erythroblastosis occurred fetal blood grouping was not performed.

All cases classified as hepatosis gravidarum had the typical clinical symptoms of itching and sometimes slight icterus. All had abnormal liver function tests. None of them had any kind of iso-immunization and the pregnancies were otherwise normal. The diagnosis, hepatosis gravidarum, used here is synonymous with benign cholestasis of pregnancy (3, 4, 15).

All fluid samples were taken by abdominal amniocentesis. Most of the sampling was part of the routine management of Rh-immunized pregnancies. A few samples were taken during elective caesarean section before opening the uterine cavity. None of the patients were in labour. No

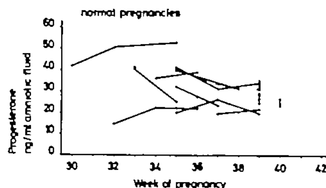


Fig 1 Amniotic fluid progesterone levels in 46 normal late pregnancies. Lines connect values from the same patient.

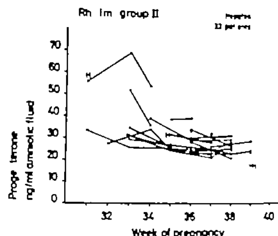


Fig 3 Amniotic fluid progesterone levels from cases with moderate erythroblastosis. Lines connect values from the same patient.

special attempt was made to locate the placenta except by simple palpation, but the puncture was always done as low in the uterus as possible The needle was directed towards the back of the fetal neck, sometimes against the region of the legs or below a moveable fetal head. A 0.8 × 80 mm needle was usually employed. Macroscopically bloodstained specimens were discarded. In 6 of the 95 Rh-negative patients a sudden rise in antibody titres occurred after the puncture. No other complications were observed.

Attempts were made to get more samples from patients with pre-eclampsia. However it was very difficult to get blood-free specimens as these patients often had a pronounced oligohydramnios.

After amniocentesis the fluid samples were centrifuged immediately and filtered through fine filter paper with the addition of a small amount of inert filterpowder (Super-cel) to remove any suspended matter The samples were then frozen and kept at −15°C until analysis.

Progesterone analyses were performed as previously described (6) Plasma or serum samples were analysed by the technique described by Johansson, 1969 (5) Parts of the fluid samples were used for other investigations which will be reported elsewhere.

RESULTS

The mean concentrations of progesterone in amniotic fluid were calculated in 4-week periods as the number of estimations in a single week was usually insufficient for statistical evaluation. Individual values for the Rh-immunized pregnancies and from the later part of 46 normal pregnancies are given in Figs. 1–5 The tendency towards higher progesterone levels in amniotic fluid for Rh groups I–III compared with normal pregnancies (Table I) is not statistically significant, but in group IV there is a significant rise during the last two 4-week periods ($0.05 > p > 0.025$ and $0.01 > p > 0.005$ respectively) Three samples with values above 100 ng/ml have been excluded. These three samples all came from pregnancies with hydropic fetuses who died of erythroblas-

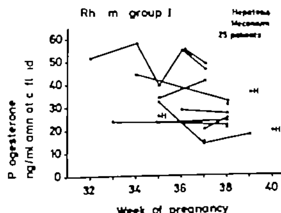


Fig 2 Amniotic fluid progesterone levels from cases with mild erythroblastosis. Lines connect values from the same patient.

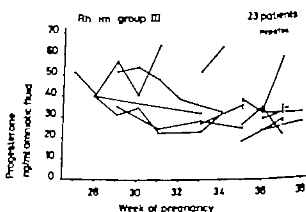


Fig 4 Amniotic fluid progesterone levels from cases with severe erythroblastosis. Lines connect values from the same patient.

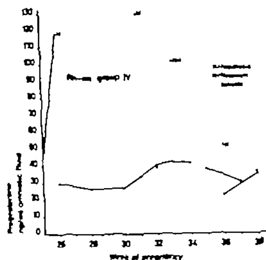


Fig 5 Amniotic fluid progesterone levels from cases with intrauterine fetal death by erythroblastosis. Lines connect values from the same patient.

tosis within few days of the sample being obtained. These liquor specimens were stained by meconium.

Determinations in plasma or serum (Table II) showed no significant difference between the Rh

Table I *Mean progesterone values in amniotic fluid (ng/ml ± S E.M.)*

Figures in brackets indicate numbers of samples

	Weeks of pregnancy		
Diagnosis	29–32	33–36	37–40
Rh group I	51 7 (1)	37 6 ± 3 3 (14)	29 7 ± 2 0 (24)
Rh group II	38 7 ± 6 6 (3)	32 5 ± 1 8 (37)	27 0 ± 0 9 (28)
Rh group III	42 0 3 5 (1)	34 1 ± 2 3 (22)	31.6 _ 2 8 (11)
Rh group IV	39 9 5 7 (5)	39 3 ± 3 1 (9)	39 6 + 7 3 (4)
Hepatosis	24 7 (1)	23 5 1 0 (3)	34 4 ± 4 8 (6)
Pre-eclampsia	40 0 (1)	33 3 (1)	39 3 (1)
Hydramnios	56 1 (1)	—	32.5 (2)
Normal pregnancies	38 9 8 0 (4)	30 3 ± 2 5 (18)	26.4 1 3 (34)

Table II *Mean progesterone values in plasma or serum (ng/ml ± S E.M.)*

Figures in brackets indicate numbers of samples

	Weeks of pregnancy		
Diagnosis	29–32	33–36	37–40
Rh group I	85.5 (1)	125.1 ± 11 7 (13)	157.5 ± 10.8 (22)
Rh group II	92.1 ± 16 4 (3)	141.5 ± 9.2 (27)	158 8 ± 10 6 (25)
Rh group III	111.2 ± 13.3 (11)	154 1 ± 9 1 (22)	147.3 ± 19.5 (8)
Rh group IV	116.2 ± 23.9 (6)	121 4 ± 19.3 (8)	198.3 ± 17.6 (6)
Hepatosis	76 6 (1)	111.9 (2)	199 8 ± 16.7 (6)
Pre-eclampsia	42.2 (1)	126.5 (1)	251.0 (1)
Hydramnios	97.6 (1)	—	188 3 (2)
Normal pregnancies	104.9 ± 7 6 (4)	134.1 ± 11.5 (16)	157 7 ± 9 7 (33)

groups and the normal pregnancies. Parallel determinations on both plasma and serum from 21 cases showed a mean serum level of 1 1.0 ± 7 9 g/ml and a mean plasma level of 128.5 ± 8.3 ng/ml. This difference is not significant.

In Table III amniotic fluid progesterone concentrations are compared with cord blood haemoglobin levels. There is a tendency for high progesterone values to be associated with low haemoglobin values and this is statistically significant ($0.025 > p > 0.01$) for haemoglobin levels of 8.0 g/100 ml or lower

Records of placental weight were available for 17 pregnancies in groups III and IV which were delivered within 7 days of sampling. The correlation factor between amniotic fluid progesterone concentration and placental weight was found t be 0.765 which is significant ($0.001 > p$). No correlation could be found betwen plasma or serum values and placental weight for these cases ($= -0.07$).

Routine liver function tests were performed on all the Rh-negative patients and 10 of them showed abnormally elevated values but only one had other linical symptoms of hepatosis.

The number of patients with hepatosis gravidarum was small (Table I) but the amniotic fluid

Table III. Progesterone in amniotic fluid (ng/ml ± S.E.M.) compared with cord blood haemoglobin

Rh-immunized pregnancies. Figures in brackets indicate numbers of samples

Cord blood haemoglobin (g/100 ml)	Weeks of pregnancy		
	29–32	33–36	37–40
> 14.1	51.7 (1)	35.2 ± 3.4 (13)	29.0 ± 1.8 (25)
14.0 – 12.1	38.7 (3)	32.1 ± [illegible].4 (24)	30.6 ± 2.1 (16)
12.0 – 10.1	—	31.5 ± [illegible].4 (13)	26.8 ± 1.2 (13)
10.0 – 8.1	36.5 ± 3.0 (12)	34.8 ± 2.0 (17)	27.0 ± 3.4 (3)
8.0 – 6.1	53.6 ± 4.4 (5)	44.3 ± 5.2 (6)	29.2 (2)
≤ 6.0	38.9 (1)	55.3 ± 13.3 (5)	37.6 ± 10.1 (3)

mean progesterone level between the 37th and 40th weeks was significantly higher than the normal mean ($0.05 > p > 0.025$). Four of the samples were stained by meconium but had rather low values of progesterone (Fig. 6). Plasma levels showed a tendency towards high values during the last weeks of pregnancy.

The diagnosis varied in the last 8 patients. Three patients with pre-eclampsia had values within the normal range as had three patients with hydramnios (Tables I and II). One patient with diabetes had progesterone levels of 4[illegible].7 ng/ml in amniotic fluid and 236.2 ng/ml in plasma in the 36th week. One woman with a twin pregnancy had levels of 36.6 ng/ml in amniotic fluid and > 220 ng/ml in plasma during the 35th week.

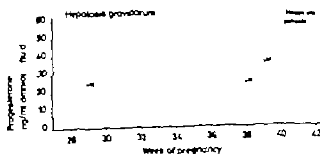


Fig. 6. Amniotic fluid progesterone levels from non-immunized patients with hepatosis gravidarum.

DISCUSSION

There appears to be no previous study of progesterone levels in pregnancies complicated by Rh-immunization. The clinical grouping of the Rh-negative patients used here largely corresponds with that used by others in connection with clinical studies of the Rh problem (1, 8, 11, 17). The cord blood haemoglobin value of 12.1 g/100 ml, used as the limit between mild and moderate disease, was obtained from our total series of amniocenteses, in 55 normal pregnancies with healthy children which yielded a mean haemoglobin value of 15.7 ± 3.6 (? S.D.) g/100 ml. As the clinical grouping of the Rh-negative patients also takes into consideration the treatment given to the children it may be somewhat subjective. This was the reason for the comparison between progesterone levels in amniotic fluid and cord blood haemoglobin given in Table III. This is in accordance with the opinion that cord blood haemoglobin is the single parameter that gives the best picture of fetal status (7). As only the most serious cases still presented any significant difference in amniotic fluid progesterone concentrations the results were the same. Estimations in plasma or serum showed less variation between the various Rh groups than did estimations in amniotic fluid and the variations were not significant. The amniotic fluid values showed a considerable overlapping between the various Rh groups.

The reason for the high values in group IV is not clear but has perhaps something to do with the observation that the placenta is very often large and oedematous in cases of serious Rh immunization. In our material from normal pregnancies there was a correlation between placental size and progesterone levels in amniotic fluid during late pregnancy (6). Also in Rh groups III and IV there was a clear correlation between amniotic fluid progesterone concentration and placental size. The meconium present in some of the group IV cases cannot have been the cause of the high progesterone content as similar meconium staining did not affect the values in the hepatosis group. In some cases with intrauterine death soon after sampling the progesterone concentrations in amniotic fluid were not so markedly high and these fetuses did not show such a marked degree of hydrops.

Several investigators have found a connection

between a high protein content in amniotic fluid and serious Rh disease (1, 11, 12) and this may indicate that penetration of substances into the amniotic cavity is facilitated in pregnancies with serious Rh disease. To some extent this may also explain the high concentration of progesterone in amniotic fluid from such cases.

Women with hepatosis had significantly higher values for progesterone in amniotic fluid compared with the normals but the number studied is small. Plasma or serum levels showed only a tendency to high values. If the women with signs of hepatic impairment among the Rh-negative patients are studied individually there seems to be a tendency towards high levels especially in plasma, compared with the other patients in the same Rh group. This trend toward high levels of progesterone in cases of hepatosis might have some connection with the observation that clinical hepatosis is associated with high plasma levels of certain steroid sulphates (14).

The few patients with pre-eclampsia in this series did not show any impairment of placental function as far as the concentration of progesterone was concerned. Also the patients with hydramnios had values within the normal range in amniotic fluid and no apparent dilution effect was observed. One of these patients had a fetus with oesophageal atresia.

The observed small variations between the various clinical groups are in line with previous findings that placental production of progesterone is largely independent of the function of the fetal part of the feto-placental unit (2, 9).

It is clear from the results that estimation of progesterone in amniotic fluid and plasma does not give much help in the evaluation of fetal status in pregnancies complicated by Rh disease. Only in the most serious cases was there a significant rise in progesterone levels, most marked when the fetus was hydropic. To be of any practical value a parameter must allow the prediction of all grades of severity of the disease, and this is not possible using amniotic fluid and plasma progesterone levels.

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THE EFFECTS AND SIDE EFFECTS OF DIURETICS IN THE PROPHYLAXIS OF TOXAEMIA OF PREGNANCY

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Abstract. In a series comprising 211 pregnant patients, the prophylactic effect of chlorthalidone on the incidence of gestosis was studied by means of a single-blind trial. The study continued from the 16th week of pregnancy until term. No beneficial effects were observed. In addition, the maternal serum potassium and sodium levels were found to be significantly lowered in the diuretic group. This made it necessary to administer potassium perorally to most of this group and to some of them even in the form of infusions. No significant differences in the parameters of the newborn infants were observed. On the other hand, the placentae in the diuretic group were definitely larger than in the control group, a fact which may be suggestive of eventual alterations in the glycogen metabolism.

Since the introduction in 1957 of the thiazides, they have been widely used in the treatment of gestosis of pregnancy. Opinions concerning their efficiency have been both positive (23, 25, 31, 17) and negative (5, 26). Their use in the prophylaxis of gestosis also has been the subject of numerous investigations, but again no agreement has been reached. Table I lists 6 series dealing with the effect of thiazides in the prophylaxis of toxaemia of pregnancy collected from the literature.

The views in regard to their usefulness are largely contradictory, but no adverse effects either on the mother or on the child have been mentioned by the writers. However, subclinical hypokalaemia and hyperuricaemia in treated mothers (18) and a slight salt and water depletion in the infants of mothers (3) who have received such treatment have been observed.

In the study presented here, an attempt has been made to elucidate the efficiency of the diuretic in question in the prophylaxis of toxaemia of pregnancy. In addition, eventual subjective sensations caused by the drug as well as electrolyte balance were followed throughout the pregnancy in a single-blind study. In connexion with the delivery certain parameters in both the mothers and the newborns were studied in order to outline the effects of the drug.

Chlorthalidone (Hygroton®), a diuretic belonging to the group of the thiazides, was selected for trial because it represents the average mode of action and efficiency of its group and has proven harmless (22) and because it possesses a comparatively protracted action which begins after 2 hours, reaches its maximum after 12 hours and persists for about 72 hours (25). This simple method of administration and an even concentration were considered essential in a mass study.

MATERIAL AND METHOD

The series consisted of 245 primigravidae from the antenatal counselling centres in Helsinki whose pregnancy had lasted for 16 weeks at the time of the beginning of this study. The mean age in the Hygroton group was 25.1 years, and in the placebo group 24.3 years. Chlorthalidone (Hygroton®), 50 mg daily, was administered to alternate patients, and the remainder received one placebo tablet per day. The tablet containers were coded so that the patients were not aware of the nature of the tablets they received.

The personnel conducting the trial could predict into which group the test subject belonged because the potassium values decreased significantly in the group receiving Hygroton. The subjects came to the ante-natal clinic every fourth week beginning in the 16th week of pregnancy. They were subjected to several investigations including tests of bodyweight, blood pressure, haematocrit, serum potassium, sodium, chloride, urea nitrogen, possible proteinuria and urinary sediment, as well as urinary bacteriological culture. At the same time, their subjective condition was registered and they were asked whether they were certain that they had remembered to take their tablets. If there was any negligence in this

Table I

Authors	No. of patients	Decreased toxaemia of pregnancy
Weseley & Douglas, 1962	267	No
Flowers et al., 1962	519	No
Cuadros & Tatum, 1964	1 771	Yes
Fallis et al., 1964	78	Yes
Finnerty & Bepko 1966	3 083	Yes
Kraus et al., 1966	1 030	No

respect, they were immediately excluded from the study. Some patients moved elsewhere during the study period and were also excluded. There were abortions, and _ patients in each group wanted to cease taking their medicine because of nausea. There were no losses from any other causes during the trial, and thus 108 patients in the Hygroton group and 103 in the placebo group completed the trial.

If the plasma potassium level decreased during the trial to 3.4 or below 500 mg of potassium chloride daily was administered to the patients until the values rose above this limit. There were 86 such cases in the Hygroton group and 3 in the placebo group. Four subjects belonging to the Hygroton group were admitted to hospital during the trial period because their serum potassium levels dropped to _.7 or less. This was corrected in a few days by means of intravenous infusions, and they continued to take their tablets both during the stationary period and after it.

Eventual cases of bacteriuria and pyelonephritis during pregnancy were treated with 6 g Sulfafurazole daily and all patients received throughout the pregnancy 1_ g of ferrous sulphate per day.

All patients included in this study were delivered in either the Helsinki University Central Hospital or in the Midwifery Institute of Helsinki. Immediately prior to

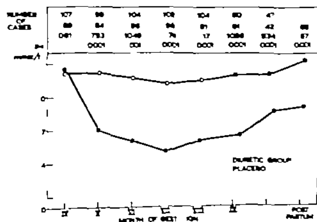


Fig. 1. Variation of the mean values of serum potassium during various months of pregnancy and following delivery in the diuretic and placebo groups; sizes of the groups, t-test variable and p values.

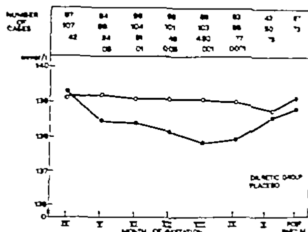


Fig. Variation of the mean values of serum sodium during various months of pregnancy and following delivery in the diuretic and placebo groups, sizes of the groups, t-test variable and p values.

delivery the investigations detailed above were repeated. In addition, the Apgar score, weight and height of the newborn, the weight and eventual calcification or infarction of the placenta, the nature and amount of amniotic fluid as well as the haematocrit and hemoglobin values and osmolality of the infant were determined.

Oedema was classified in this study as follows: mild (slight depression in the subcutaneous tissues covering tibia following pressure), moderate (deep depression on the tibial surface) and severe (universal oedema, including the face). In the present study only the latter two groups were recorded as having oedema. In the proteinuria group those cases were included in which the 24-hour urinary excretion of protein exceeded 0.4 g. Blood pressure was measured in the recumbent position, and the highest and lowest values measured during the last 1 hours preceding delivery were included in the study. The weight increase refers to the difference in weight between the date of delivery and 16th week of pregnancy. Blood samples for electrolyte determinations were taken immediately prior to delivery, usually in the morning. The newborn infants were measured immediately after birth, and blood samples were taken on the same day. The Apgar score was determined 5 min following birth.

RESULTS

With the exception of _ subjects in both groups who wanted to drop out of the study because of nausea, side effects were not very serious in either of the groups. However 20 subjects in the diuretic group complained of nausea, 4 had experienced nasal or aural congestion, intense headache and 2 dizziness or faintness. Three patients in the control group complained of nausea, one of intense headache. In the latter group there were _ cases of anaemia (Hb < 9 g/100 ml) because

of hich the patients had to be admitted to the hospital in the middle of their pregnancy

When the electrolyte levels were followed during pregnancy there were significant differences between the groups in regard to both potassium and sodium (Figs. 1 and 2).

As far as potassium was concerned, the differences were significant throughout the pregnancy The differences between the groups were most marked between the 4th and 9th months of pregnancy and during this period there was also a slight decrease in levels in the control group. In the case of sodium, the differences between the groups were only significant from the 6th to 9th months of pregnancy The serum levels of sodium in the control group remained fairly constant throughout the pregnancy The differences bet een the groups were clearly smaller than in the case of potassium.

When the serum concentrations of urea nitrogen were compared, the differences between the groups were obvious, the values being higher in the diuretic group. Only at the time of delivery did this difference level off to almost significant (Fig. 3).

Similarly the haematocrit values were higher in the diuretic group, although without statistical significance except during the 6th to 9th months of pregnancy when the differences were almost significant (Fig. 4).

There were definit differences in body weight as well as in serum chloride concentration.

On the other hand no differences were observed

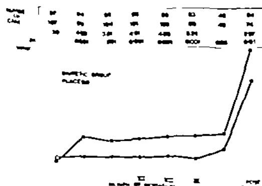


Fig. 3 Variation of the mean values of serum urea-N during arious months of pregnancy and following delivery in the diuretic and placebo groups; sizes of the groups, test ariable and *p* values.

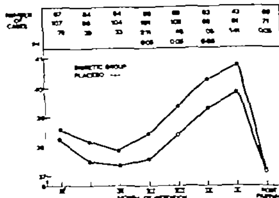


Fig. 4 Variation of the mean hematocrit values during various months of pregnancy and following delivery in the diuretic and placebo groups; sizes of the groups, *t*-test variable and *p* values.

in blood pressure, degree of proteinuria, urinary sediments, or bacterial content of the urine.

The observations made on the day of delivery are shown in Table II.

Although the maternal weight gain was significantly greater in the control group, no significant difference was observed in the incidence of oedema. Similarly there were no differences in blood pressure. Proteinuria occurred significantly more often in the diuretic group The values of potassium, sodium and chloride were significantly lower in the diuretic group. On the other hand, the haematocrit and urea nitrogen alues were clearly higher

The erage weight and height of the infants

Table II. *Mean values f maternal parameters on the day of delivery in the diuretic and control groups as ell as the significance of the differences*

	Diuretic group	*p*	Control group
Edema,	18.2	> 0 05	18 4
Proteinuria, %	5.6	0 001	1 9
Systolic blood pressure, mm Hg	120–135	0 05	121 135
Diastolic blood pressure, mm Hg	79–90	> 0 05	79–89
Weight gain, kg	7 8	0 001	9 7
Potassium, mmol 1	3.76	0 001	4 19
Sodium, mmol 1	138.1	0 001	139 1
Chloride, mmol ml	101.26	0 001	103.3
Haemoglobin, g 1	12.4	0 05	12.2
Hematocrit cl,1	40 4	0 05	39 5
Urea-N, mmol 1	10 19	0 001	8.15

Table III. Mean values of neonatal parameters on the day of birth in the diuretic and control groups as well as the significance of the differences

	Diuretic group	p	Control group
Weight, g	3 557	<0.05	3 391
Height, cm	50.5	<0.05	49.7
Apgar score <7"	8.69	>0.05	8.76
Osmolality, mOsm/kg	292.9	>0.05	292.2
Hemoglobin, g/l	15.9	>0.05	15.4
Hematocrit, cl/l	50.2	>0.05	49.9
Placental weight, g	651.4	<0.001	585.3
Placenta/infant weight, %	18.4	<0.01	17.3

were significantly greater in the diuretic group (Table III). There was no significant difference in the condition of infants. No deaths occurred in the entire series. The differences in the haemoglobin and haematocrit values and in osmolality were insignificant. The differences in the placental weights were significant. There was a significant difference in the foetal/placental weight ratio between the two groups. Whilst the infants in the diuretic group were larger, their placentae were proportionately larger still. The standard deviations in the relations between the placental and infant weights were 2.97% in the diuretic group and 2.68% in the control group, being thereby somewhat higher in the former.

There were no differences in the amount or nature of amniotic fluid, placental calcification or number of infarctions (38 out of 107 in the diuretic group and 34 out of 102 in the placebo group).

DISCUSSION

In the present study no significant difference in terms of incidence of toxaemia was found between the groups. There was one exception, namely proteinuria, which was more frequent in the study series. However, the number of cases was very small.

It is surprising that clinical oedema occurred in both groups with just the same frequency, although both the smaller increase in weight and the haematocrit and urea nitrogen values suggest a moderate dehydration in the diuretic group. It is probable that, in the hospitals, insufficient attention is paid to oedema. It should also be taken into account that the diagnosis of oedema by the traditional methods is in no way satisfactory and requires methodological improvement, if any attempt to attain the same exactness as in assessment of the other components of toxaemia of pregnancy.

It has previously been demonstrated that the presence of oedema definitely increases the size of the newborn infants and improves their prognosis (32, 33). Therefore the observation made in the present study that the children in the diuretic group were bigger in size in comparison with the control group is contrary to the expectations. It is true, however, that this observation was not very highly significant.

As to the pathogenesis of toxaemia of pregnancy, it is recognised that twin pregnancy, polyhydramnios, sclerosis associated with increasing maternal age, or some other factor may result in ischaemia of the uterus which causes a rise in blood pressure (4, 22). It is probable that this *is due to release of renin from the placenta*. This splits a fragment away from angiotensin, which is an alfa$_2$-globulin, and results in the formation of angiotensin I which, in turn, is split to angiotensin II. This, unlike angiotensin I, is a biologically active agent, an efficient vasopressor which also increases the amount of aldosterone in the blood. During pregnancy it is normally present in five-fold concentrations (34), although mainly in protein-bound form and this may cause loss of potassium and increase in oedema.

An elevated plasma potassium concentration reduces secretion of renin (37), whereas chronic deficiency of potassium stimulates formation of renin (1). It might be supposed that aldosterone antagonists would have a beneficial effect in the treatment of toxaemia of pregnancy, but this has not been proved to be the case, although definite increases have been effected in sodium and water excretion (3). This is also supported by the fact that the concentration of aldosterone and even that of renin has been found to be lower in patients with gestosis than in healthy pregnant women (35). However, it is possible that the concentration of these hormones is low because they are metabolized at a greater rate in toxaemia of pregnancy. It is also possible that the target organs are more sensitive to small amounts of angiotensin (2).

It has been found that when the gestosis be-

comes more severe the total body water becomes reduced (20) and sodium is possibly implicated in this phenomenon. On the other hand sodium depletion induced by deprivation of dietary sodium alone or in a combination with natriuretic agents will consistently produce increased renin and aldosterone release, and a sodium load will suppress the secretion of renin and aldosterone (6 9 29) Also in the present work, sodium depletion induced by diuretics was found to be considerable especially towards the end of pregnancy which probably is related to the marked dehydration observed. The copious potassium depletion speaks for increased aldosterone synthesis, which also may be accompanied by activation of the renin-angiotensin system. Howere, no differences were observed in blood pressures.

The old concept of beneficial effect of bed rest on hypertension during toxaemia of pregnancy seems to acquire a theoretical basis. The change in posture from supine to upright is potent stimulus for renin release (8 27), and sodium depletion seems to sensitize this mechanism (28) Without being aware of this observation, all blood pressure determinations in the present work were performed in recumbent patients.

In the present study the infants delivered were slightly significantly bigger in the diuretic than in the control group. In contrast, the placentae in the former group were highly significantly heavier This becomes most clearly evident when comparing the foetal/placental weight ratio, which shows a definite increase. This observation (9 11) strongly suggests a diabetogenic property of diuretics which has already been reported in numerous other papers and which is also supported by the difference prevailing in regard to the birth weights of the infants So far observations are available to show whether placental weights correlate with the existence of latent diabetes On the basis of the present study it could be estimated that this correlation could be better than that between the weights of the newborn and latent diabetes, which has been employed until so far

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Table III. *Mean values of neonatal parameters on the day of birth in the diuretic and control groups as well as the significance of the differences*

	Diuretic group	p	Control group
Weight, g	3 557	<0.05	3 391
Height cm	50.5	<0.05	49 7
Apgar score <7*	8.69	>0.05	8.76
Osmolality mOsm/kg	292.9	>0.05	292.2
Hemoglobin, g/l	15.9	>0 05	15 4
Hematocrit cl/l	50.2	>0.05	49 9
Placental weight, g	631 4	<0.001	585.3
Placenta/Infant weight, %	18 4	<0.01	17.3

were significantly greater in the diuretic group (Table III) There was no significant difference in the condition of infants. No deaths occurred in the entire series. The differences in the haemoglobin and haematocrit values and in osmolality were insignificant. The differences in the placental weights were significant. There was a significant difference in the foetal/placental weight ratio between the two groups. Whilst the infants in the diuretic group were larger their placentae were proportionately larger still. The standard deviations in the relations between the placental and infant weights were 2.97% in the diuretic group and 2.68% in the control group, being thereby somewhat higher in the former

There were no differences in the amount or nature of amniotic fluid, placental calcification or number of infarctions (38 out of 107 in the diuretic group and 34 out of 102 in the placebo group)

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THE AMOUNT AND FORM OF RADIOACTIVITY IN HUMAN MILK AFTER LUNG SCANNING RENOGRAPHY AND PLACENTAL LOCALIZATION BY ^{131}I LABELLED TRACERS

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Abstract The secretion of radioisotope into mothers' milk was studied after the administration of carriers labelled with ^{131}I. The radiopharmaceuticals used were 300 μCi macroaggregated human serum albumin, in short MAA (for lung scanning), 25 μCi ortho-iodohippuric acid, in short OIH (for renography) and 10 μCi human serum albumin, in short HSA (for placental localization) In the MAA series (7 patients) the maximum radioactivity secreted into milk in the 24 hours following injection was 0.7%, and the mean activity 0.14% of the injected dose The corresponding figures of the OIH series (25 patients) were 2.4 and 0.32%. In both cases activity was at its maximum during the first 24 hours after injection In the HSA series (8 patients) the maximum radioactivity secreted into the milk during 24 hours after injection was 0.22% of the injected dose After the administration of OIH and apparently also of MAA, the radioactivity secreted in 24 hours increases if the excreted volume of milk increases.

The radio-iodine secreted in the milk was not bound to the radiopharmaceutical originally administered, but was mainly in the form of free isotope.

The breast feeding of babies whose mothers had undergone lung scanning, renography or placental localization was discussed It was proposed that milk should be discarded for a maximum of 7 days following lung scanning and a minimum of 2 days following renography provided the examinations were carried out by means of the radiopharmaceuticals and doses used by the present authors.

In the past ten years, the introduction of new methods, as well as the more extensive application of existing methods, has greatly increased the use of radioisotopes in diagnosis. The result has been that increasing numbers of people are exposed both to direct radiation, and to indirect radiation which is even more difficult to control. The exposure of patients examined with radioisotopes is relatively well described in numerous reports, whereas indirect exposure in connection with various isotope examinations has received less attention.

The purpose of the present study was to investigate the amount of radioactivity secreted into the milk of patients who post partum were subjected to lung scanning or renography or ante partum to placental localization. In addition, we sought to discover whether the isotope is excreted in the same form as that in which it was injected.

MATERIAL AND METHODS

Because of suspected pulmonary embolism, 7 mothers underwent lung scanning post partum with 300 μCi macroaggregated human serum albumin labelled with ^{131}I, in short MAA (Institutt for Atomenergi, Kjeller, Norway) The ^{131}I content of the milk was determined for a few days after the injection.

Following renal dysfunction during pregnancy 25 mothers were subjected to renography 1–15 days (in most cases 2–5 days) post partum. The dose used for examination was 25 μCi ortho-iodohippuric acid labelled with ^{131}I, in short OIH (N. V Philips-Duphar Cyclotron and Isotope Laboratories, Holland). The ^{131}I content of the milk was determined twice in 72 hours, usually 24 and 72 hours after the injection.

The localization of the placenta of 8 patients was carried out using 10 μCi human serum albumin labelled with ^{131}I, in short HSA (Institutt for Atomenergi, Kjeller, Norway). The ^{131}I content of the milk was determined 4–16 days after the injection and 4–8 days after delivery once for each patient

In all cases, the ^{131}I content of the milk during a 24-hour period was determined by routine methods using a standard. The milk volume was measured to determine the total activity excreted in the milk during the 24 hours.

The time between manufacture and administration of

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the second, and 264 ml during the third. The total excreted radioactivity and the radioactive concentrations after OIH injection were maximal in the milk of the first 24 hours. At this period the highest activity excreted in any individual was ..4% of the injected activity and the mean value of the excreted activity of 25 patients was 0.32%.

The results for the first day after OIH injection were divided into three groups according to the volume of milk excreted. (I) excretion $\leq$ 100 ml, (II) excretion 100–200 ml, and (III) excretion $\geq$ 200 ml. It was seen that both the total activity excreted and the radioactive concentration in the milk increased in proportion to the milk volume. By means of the *t*-test, a statistically significant difference was noted between groups I and II ($p < 0.001$) and between groups I and III ($p < 0.05$). The mean total activities and radioactive concentrations (± 1 S.D.) corresponding to the mean values of the groups are shown in Fig. 3. No similar differences could be shown in the second and third 24-hour periods. This was probably because the secretion of milk had by then become stabilized at about 300 ml, and in the two cases remained under 100 ml.

After the administration of HSA the highest concentration of radioactivity 0.10 μCi/l (milk excretion 110 ml/24 hours) was recorded five days after injection and four days after the delivery. The maximum radioactivity excreted in the milk during 24 hours, 0.022 μCi (milk excretion 230 ml/24 hours) was recorded in another patient six days after injection and five days after delivery. This excreted radioactivity therefore was 0.22% of the injected activity. In the other patients, both the total radioactivity excreted in milk and the concentrations of radioactivity in the milk were lower.

The form of the radioactivity excreted

Electrophoresis revealed that radioactivity in milk after the administration of MAA or HSA was not bound to any protein fraction. Control electrophoresis with the same tracers showed that the greater part of the radioactivity contained in tracer albumins was localized in the albumin fraction. Thus the radioiodine is not excreted into the milk in its tracer albumins.

When the excretion of *p*-aminohippuric acid

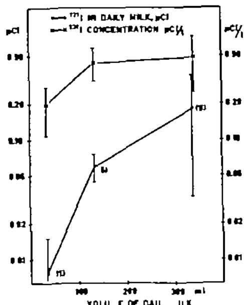


Fig. 3. Correlation between the ^{131}I concentration and the total activity in milk excreted in the first 24 hours after the administration of *o*-^{131}I-hippuric acid and the volume of the milk excreted. The patients were divided into three groups according to the volume of milk excreted. (I) 100 ml, (II) 100–200 ml, (III) 200 ml. The dots indicate the mean ^{131}I concentrations and excreted activities in the milk corresponding to the mean volumes of milk excretion per group (± 1 S.D.).

was tested, an average of 96.9% of the dose injected in three patients was excreted with the urine during the 24 hours following a single injection, whereas no PAH nor acetylated PAH was recorded in the milk collected during the same 24 hours. Since the sensitivity of the method by which PAH is measured is 1.0 μg/ml, it is evident that the milk of these three patients must have contained less than 0.26% of the PAH dose calculated per liter of milk. The corresponding mean excretion of radioactivity after the administration of OIH in 25 patients was 1.4% per liter of milk.

Neither PAH nor acetylated PAH was found in the milk excreted during the infusion of PAH at a steady rate for 120 min.

DISCUSSION AND CONCLUSIONS

The present series contained only one patient whose milk excretion after the administration of MAA amounted to 300–350 ml per 24 hours

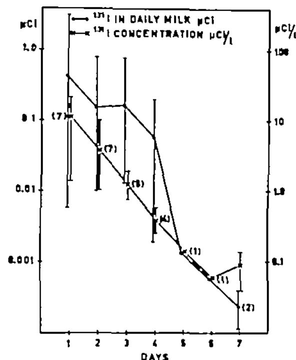


Fig 1 The daily mean values of the ¹³¹I concentrations in and the total activity excreted with the 4-hour milk flow after the administration of 300 µCi macroaggregated human serum albumin labelled with ¹³¹I The line segments accompanying the dots illustrate the total range of dispersal of the results obtained. The number of patients is given in brackets.

the tracer was not recorded in any f the investigations, but it could never have exceeded 15 days.

To block the thyroid, patients receiving MAA or HSA were given 10 drops 10% potassium iodide solution on the day before, on the day of and on the day after the examination.

To discover whether or not the radioiodine secreted into the milk after the administration of MAA or HSA was bound to milk proteins, an examination by electrophoresis was carried out both on cellulose acetate membranes and Whatman 3 M+M paper (pH 8 6, 0.1 M barbital buffer) and the radioactivities of the fractions were measured. Macroaggregated and plain human serum albumin labelled with ¹³¹I served as controls f r the lectrophoretic runs.

An ttempt was also made to study the secretion, if any of hippuric acid into milk after administration of OIH using only para-aminohippuric acid (PAH) in a 0.9% solution f sodium chloride and assuming that PAH and o-¹³¹I-hippuric acid behave simila ly in the organism. Single intravenous injections, erage 384 mg each, of PAH (Sodium Aminohippurate Merck, Sharp & Dohme, USA) were given to three patients. In addition, PAH was infused into one patient at a teady rate f 170 min, with plasma concentration of 3.0 mg/100 ml (total 640 mg) The PAH was determined by the method of Smith et al. (7). In order to verify the acetylated PAH (3), milk was heated together with HCl (final concentration of HCl 3.0 M) for 60 min, and the PAH was determined as above. Determinations were also carried out on urine and plasma.

RESULTS

The amount of the radioactivity excreted

As regards time the fall in the ¹³¹I content of milk excreted after the administration of MAA approximately followed a simple exponential function during the few days observation. In about 48 hours it fell to one-tenth of the initial content. The daily total activity excreted in the milk showed considerable variation (Fig. 1) The total excreted radioactivity and concentrations of radioactivity in the milk were at their maximum during the first 24 hours after injection. At this period the maximum activity recorded for a patient was 0 7% of the injected activity and the mean value of the excreted activity of 7 patients was 0.14%

The mean ¹³¹I content and mean radioactivity excreted in the milk on three consecutive days following the administration of OIH are shown in Fig. 2. The mean volumes of excreted milk were 164 ml during the first day 384 ml during

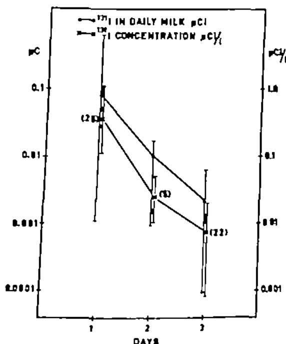


Fig 2 The three daily mean alues of the ¹³¹I concentrations in and the total activities excreted with the 4-hour milk flow after the administration of 25 µCi o-¹³¹I-hippuric acid. The line segments accompanying the dot show the total range of dispersal of the results obtained. The number of patients is given in brackets.

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during the four-day period of examination. All the other patients excreted less than 100 ml milk per 24 hours. On the other hand, the mean milk excretion of patients in the first two days after the MAA injection was about 50 ml, in the next two days about 100 ml, and later 5–10 ml. These facts probably caused the irregular course of the mean value curve of radioiodine excretion per 24 hours with "peaks" on the third and fourth days (Fig. 1) It seems obvious that after the MAA injection the amount of radioiodine excreted with the milk during 24 hours increases when the excreted milk volume increases, as was also shown after the OIH injection. Unless as a result of illness or for some other reason, the milk excretion is subnormal it also seems evident that radioactivity excreted in milk may exceed the average found the present series.

In the present OIH series the mean radioactivity excreted in milk in the first 24 hours after the OIH injection (0.32% of the injected activity) was lower than that reported by Schwartz et al. (5) This, in our opinion, is at least partly due to the fact that the mean volume of milk excreted in the present series was approximately half that in their material. The value reported by Hengst (2) was also higher however in his studies he had used both *o*-iodohippuric acid and chloriodine propyl inulin the results are therefore not fully comparable.

In an individual case after the OIH injection, the activity excreted in the milk during a 24-hour period was higher (2.4% of the injected dose) than in any other case The cause could not be traced Similar values have also been quoted by other authors (2, 5)

After the HSA injection, the maximum activity excreted in the milk during a 24-hours period was, in present series, 0 022 μCi (0.22% of the injected dose) obtained immediately after the milk flow started. It is obvious that if the milk flow exceptionally starts immediately after delivery and the interval between the HSA injection and delivery is very short, activity excreted in the milk may be significantly higher

According to these studies it seems evident that, in all three methods used, radioactivity in the form of "free iodine" is excreted in the milk without its carrier agents, albumin and ortho-iodohippuric acid, having passed into the milk. When milk is used to feed the baby the radioiodine as free isotope is readily taken up by the infant thyroid gland, whereas had it been in a bound form, the affinity would be less or absent.

The results presented reveal that radioactivity after the use of macroaggregated human serum albumin, ortho-iodohippuric acid or plain human serum albumin, labelled with ^{131}I is excreted into the milk of lactating mothers. According to different authors, the irradiation dose entering a child's thyroid after the administration of ionic radioiodine ^{131}I depends on the thyroid size, iodine accumulation and effective half-life and amounts to 25–32 rad per microcurie administered (1 6) On the other hand, according to ICRP recommendations, the irradiation dose for a child's thyroid should not exceed 1.5 rad per year (4) This means that the total ^{131}I activity in radioiodine-containing milk given to a child should not exceed 0 05 μCi per year

When the total radioactivity transmitted to a child's thyroid is estimated according to the maximum activity excreted in milk after lung scanning, renography and placental localization, and the doses used are those of the present study we feel that the use of the milk of lactating mothers should be limited as follows:

(*a*) after lung scanning the milk should be discarded for not less than seven days,

(*b*) after renography the corresponding period should be not less than two days,

(*c*) after placental localization the amount of radioactivity in the milk seems small and apparently does not debar the milk from being used to feed babies.

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BRENNER TUMOURS

Oestrogen Activity and Coincidence with other Neoplasms

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Abstract Six Brenner tumours diagnosed in 1962–1970, of hich at least four ere accompanied by increased oestrogen activity are described. All the tumours were small, 5–20 mm in diameter, and in addition to the Brenner tumour there ere other genital tumours; endometrial carcinoma, granulosa-cell carcinoma, thecoma, mucinous cystoma in four cases. The coincidence of Brenner tumour with increased oestrogen activity and with other tumours is discussed

Brenner tumour is   fairly uncommon ovarian neoplasm. There are no typical symptoms and a small tumour may be asymptomatic for years. A total of some 500 cases has been reported to date (Novak & Woodruff 1967) These studies have generally dealt with the occurrence, pathology and histogenesis of the tumour (von Numers, 1945 Woodruff & Acosta, 1962) It has been known that a Brenner tumour may sometimes secrete oestrogens (Schiffmann, 1932 Jonas, 1959 Shaaban et al., 1960 Ming & Goldman, 1962) On the basis of histologic study of the endometrium, it has been estimated that this type of Brenner tumour accounts for about 7.5% of ll cases (Farrar et al 1960).

Six patients with Brenner tumours included in the present study were treated t the Department of Obstetrics and Gynaecology University Central Hospital, Turku, in 1962–1970 Because of the supposedly closer correlation between Brenner tumours and oestrogen activity than estimated in the literature, it seemed necessary to draw attention to this syndrome.

CASE REPORTS

Case 1 The patient was admitted in 196_ because of tumour in the lower abdomen She was 72 at the time, and since her menopause 23 years earlier had had no bleeding. At operation   smooth-surfaced ovarian tumour about 15 cm in diameter was seen on the right. The left ovary and the uterus ere normal and of fairly small size. Bilateral oophorectomy was performed, the uterus as not removed. When the tumour was split two fairly large cysts filled ith greenish fluid ere seen in it. A little of the tumour was solid tissue hich was pale on its cut surface.

Histologic examination revealed   benign mucinous cystadenoma ith an almost solid area 2 mm in diameter on one edge. Structure as typical of   microcystic Brenner tumour Sharply defined epithelial islets in the middle of the dense stroma of fibroma type were observed in this area. The right ovary was atrophic. No specimen from the uterus was available for histologic study

Case 2 The patient as admitted in 1964 because of haemorrhage from the genitalia. She was 58 at the time and had had her menopause eight years earlier Diabetes mellitus had been diagnosed in 1963. Curettage was per formed The histologic diagnosis was endometrial adenocarcinoma; there was no normal endometrium in the specimen Treatment consisted initially of two applications of radium followed by hysterectomy and bilateral oophorectomy The uterus was   little larger than normal and the endometrium was thin after the radiation. Both ovaries were small, there as   small solid tumour on the surface of the right ovary

The first node detected in the right ovary proved on histologic examination to be   cortical, microcystic well-demarcated Brenner tumour 5 mm in diameter A few atretic follicles were also seen The lef ovary was atrophic The endometrial specimen displayed only necrosis after the radiation.

Case 3 The patient as admitted in 1967 because of scanty bleeding hich had continued for about   month. She as 90 at the time and had had her menopause fi years earlier A tumour as palpated in the left lower abdomen and the patient as operated on Hysterectomy and bilateral oophorectomy were performed At the operation   solid ovarian tumour about 10 cm in diameter was detected on the left, and in the right ovary   small solid tumour was seen. The uterus as normal, fairly small in size.

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Fig 1 Case 4 Microcystic epithelial structure in a small Brenner tumour (hematoxylin-eosin, × 90).

Hist logic examination showed the larger tumour in the left ovary to be thecoma, atretic follicles were seen in addition. The right ovary displayed on the boundary between the cortex and medulla an almost solid microcystic Brenner tumour 20 mm in diameter The myometrium, in which a small myoma was observed, was not atrophic and in the endometrium there was distinct evidence of oestrogen effect.

Case 4 In 1958, when the patient was 34 the first curettage was undertaken for prolonged, massive bleeding. The histologic diagnosis was cystic hyperplasia of the endometrium. Curettage proved necessary again in 1965 and 1966, and endometrial hyperplasia was established on both occasions. The patient was given progesterone therapy after the curettage. A fourth curettage was performed in 1968 for bleeding of long duration, and histologic examination now revealed cystic hyperplasia with slight adenomatous changes of possibly premalignant p-pearance (Fig. ?).

At the operation one month later resection of the right ovary and left salpingo-oophorectomy were performed. The right ovary was enlarged to about 5 cm in length and cystic, the left was about normal in size. Small hemorrhagic cysts were seen on the cut surface of this ovary and in its centre a pale, solid area about 10 mm in diameter The uterus was larger than normal and the endometrium thick.

Histologic examination revealed in the cortex of the left ovary a typical microcystic Brenner tumour 10 mm in diameter (Fig. 1) which was sharply demarcated from its environment. I addition, an acth follicle was encountered in the left ovary Specimens taken from the right ovary displayed a cystic atretic follicle. The endometrial specimen again displayed mild cystic hyperplasia with slight adenomatous changes.

Case 5 The patient was admitted in 1969 because of haemorrhage She was 72 at the time and had had her menopause — years earlier Curettage had been performed for bleeding in 1962 and 1964 and the histological diagnosis on both occasions had been endometrial hyperplasia. Carcinoma of the left mammary gland had been diagnosed year earlier for which the therapy had been mastectomy and radiotherapy The histologic diagnosis of the curettage specimen was again cy tic hyperplasia f the endometrium. A tumour was detected now in the right adnexa. At oper tion a soft well vascularised ovarian tumour about 7 cm in diameter was found. The left ovary was normal on gross examination. The uterus was the size of fist and had several intramural myomas. Subtotal hysterectomy nd bilateral oophorectomy were performed.

The tumour removed from the right ovary was histologically fairly solid but partly haemorrhagic and necrotic granulosacell carcinoma. I places tumour tissue had pread outside the capsule and the tumour was pparently malignant. The left ovary displayed on the boundary between the corte and medulla, in a w ll-demarcated area 7 mm in diameter typical microcystic, almost solid Brenner tumour The uterine specimen revealed myomatous tissue and h perplastic endometrium.

Case 6 The p tient was admi ted in 1970 because of scanty bleeding She was 70 nd had had her menopause 1 years arlier A tumour was found in the lower abdomen. At operation cystic fairly soft ovarian tumour



Fig. 2. Case 4. Recurrent slight cystic hyperplasia of the endometrium over period of 10 years in 44-year-old patient ith slight glandular atypia (hematoxylin-eosin, 90).

about 10 cm in diameter containing serous fluid was detected on the left.

Histologically part of the cysts ere paroovarian cysts in the hilus of the ovary. On the boundary of the cortex and medulla cystic Brenner tumour 20 mm in diameter was seen. In some cysts the epithelium as cuboid, mucin secreting. The right ovary was atrophic with some atretic follicles. The endometrium was thin but the glands displayed distinct oestrogen effect.

The cases reported in the foregoing are summarized in Table I.

DISCUSSION

Contrary to earlier opinion, Brenner tumours are obviously often associated with elevated oestrogen activity. Three cases displayed postmenopausally a distinct oestrogen effect on the endometrium, and one case involved recurrent cystic hyperplasia in the fertile age. One patient had in addition to a Brenner tumour endometrial adenocarcinoma which is known to be frequently associated with increased oestrogen production. No histologic specimen from the uterus was available in one case in which Brenner tumour was seen together with mucinous cystadenoma.

The small size of the Brenner tumours in all the cases also attracts attention. The tumours were 5–20 mm in diameter. A tumour of this type might also be regarded as a certain kind of

Table I. *Some details of the cases*

Cases	Age	Abnormal bleeding	Endometrium	Other tumours
1 9249/62	72		—	Mucinous cystadenoma
2 11573/64	58		Adenocarcinoma	Myomata
3 7884/67	50		Postmenopausal oestrogen effect	Thecoma
4 5846/68	44		Cystic hyperplasia	Atretic follicular cysts
5 2477/69	72		Cystic hyperplasia	Granulosa cell carcinoma, myomata uteri, carcinoma of breast
6 5673/70	70		Postmenopausal oestrogen effect	Mucinous cystadenoma

secondary reaction in tissue to continuous long term oestrogen stimulation and the tumour itself need not necessarily have secretory activity Persistent trophic irritation might in the manner of endometrial carcinoma lead even to malignant degeneration This is however rare (v Numers, 1945) The other neoplasms in our material, granulosa-cell tumour thecoma, mammary carcinoma (Grönroos & Aho 1968) myomas and mucinous cystadenoma might be associated with elevated oestrogen activity either as a primary source or as a target organ Recurrent anovulatory menstrual cycles were an additional finding in one of the cases. Thus, abnormalities in oestrogen metabolism are often associated with Brenner tumours and this problem requires additional study

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FETO MATERNAL HAEMORRHAGE IN ECTOPIC PREGNANCY

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Abstract. Feto-maternal transfusion was found to be significantly more common in ectopic pregnancy than in normal pregnancy of corresponding duration. Ectopic pregnancy but never normal pregnancy in the first trimester, is sometimes associated with feto-maternal transfusion sufficiently large to cause Rh-immunization.

It is concluded that an ectopic pregnancy in Rh-negative non-immunized women is an indication for prophylaxis with anti-D-gammaglobulin, administered as early as possible after the operation.

METHODS AND MATERIAL

The method of Kleihauer et al. (5) was used for demonstrating fetal blood cells in the maternal circulation. Capillary blood samples were obtained from each patient and two smears were prepared for examination. The smears were afterwards examined and cells in 50 low-power fields were counted. This means that about 150 000 maternal blood cells were counted. The number of fetal cells per 50 low-power fields was then expressed as described by Woodrow et al. (12). Only those cases in which at least one fetal cell was traced in each of the two smears were regarded as positive.

The series consisted of two groups of women. Group I was made up of 59 pregnant women in the 10–12th week of apparently normal pregnancy. Group II contained 40 women with ectopic implantation of the fertilized ovum which was subsequently confirmed histologically. In this group the women were approximately 8 to 1[illegible] weeks pregnant. All the women in Group II, except three, had been subjected to salpingectomy and blood had been found in the abdominal cavity in all except two. In the ectopic group the blood sample was obtained 3 to 12 hours after laparotomy in 36 and more than 12 hours after the operation in 4 cases. The proportion of Rh-negative women was the same in both groups.

RESULTS

The frequency of positive samples in Group I was 8.5%, a figure in good agreement with that found by other investigators (3). The corresponding figure for group II, however, was 32.5%. The difference between the groups was significant ($\chi^2 = 10.98$, $P = 0.001$). The distribution of positive samples according to the number of fetal blood cells per 50 low power fields is given in Table I. None of the women in group I had more than 5 fetal cells per 50 low power field compared with 4 in group II (Table II). In these four last-mentioned cases more than 0.2 ml fetal blood must have passed into the maternal circulation (12).

DISCUSSION

Rh antigen develops within the first 6 weeks of life of the fetus (10) but the strength and viability of the antigen in such an early phase of pregnancy has been questioned (9). One case of immunisation by the 15th week of pregnancy is, however, on record (4). Opinions differ about the amount of blood necessary for the induction of immunisation. Zipursky et al. (13) have shown that primary immunisation requires 1.3 ml, while 0.1 ml is sufficient to produce a secondary antibody response. Later, however, they also found that repeated doses of 0.1 mg of Rh-positive blood immunised Rh-negative volunteers (14). In a leading article in the British Medical Journal (1)

Table I. *The presence of fetal cells in normal and ectopic pregnancy*

	Pos.	Neg.	No. of cases	Incidence (%)
Group I	5	54	59	8.5
Group II	13	27	40	32.5

$\chi^2 = 10.98$, $P = 0.001$

Table II *Estimation of fetal score*

	0	1	>1	>5	No. of cases
Group I	54	3	2	0	59
Group II	27	4	5	4	40

the smallest amount of blood capable of producing immunisation was given as 0.5 ml. Most investigators, however feel that a much smaller volume is sufficient. The significance of spontaneous abortion in the "sensibilisation" of a non-immunised Rh-negative woman who is found to be immunised in her next pregnancy is dubious, since a small percentage of Rh-negative women can apparently become immunised during their first fullterm pregnancy without any known previous stimulus.

In provoked abortion (legal or illegal) on the other hand, the risk of feto-maternal transfusion is probably considerable (7) Cases of Rh-immunisation after legal abortion have been reported (8)

The present investigation showed that ectopic pregnancy also involves a serious risk of feto-maternal transfusion In 4 of the cases reported the transfusion was sufficiently large to induce Rh immunisation. It must therefore be recommended that prophylactic anti D-gamma-globulin be given to non-immunised Rh-negative women who have just had an operation for ectopic implantation of a fertilised ovum

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INDUCTION OF THE "PREGNANCY ZONE" PROTEIN BY ORAL CONTRACEPTIVES

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Abstract The "pregnancy zone" serum protein was found in 73 out of 145 women (50%) taking oral contraceptive drugs. There was significant difference with respect to the frequency with which the "pregnancy zone" was induced by different types of contraceptives. Conlutett produced the "pregnancy zone" in 64% of the women and Follinyl in 38%. This difference may reflect the composition of the pills. A history of previous pregnancies or the duration of administration of the hormones had no significant effect on the induction of the "pregnancy zone".

Smithies (5), using starch gel electrophoresis, was the first to describe the "pregnancy zone" protein in the sera of pregnant women. This protein has been found from the ninth week of pregnancy with increasing frequency up to the twenty-fifth week, when it was present in 81% of all pregnant women as judged by starch gel electrophoresis (1). De Alvarez & Afonso (2) demonstrated that the "pregnancy zone" was induced by the oral contraceptive drug Enovid (10 mg norethynodrel and 0.15 mg mestranol) in about 90% of the women after t o cycles of administration. Beckman et al (3) observed that the "pregnancy zone" occurred in significantly higher frequency among women carrying female fetus. The reason for this is not clear.

The purpose of the present investigation was to confirm the previous results by de Alvarez & Afonso (2) to establish if the "pregnancy zone" protein may be produced after administration of common oral contraceptive drugs with a composition different from that of Enovid, and to lucidate the effects of different kinds of pills, the duration of administration and previous pregnancies.

MATERIAL AND METHODS

Blood samples ere obtained from series of 218 healthy women attending the contraceptive clinic at the Department of Obstetrics and Gynaecology University of Umeå. Of these 218 omen 145 ere already taking oral contraceptives, while 73 were coming for prescription. All omen attending the clinic, were asked to participate in the investigation. About 5% refused for various easons.

The presence of the "pregnancy zone" was determined by electrophoresis in starch gel according to the technique

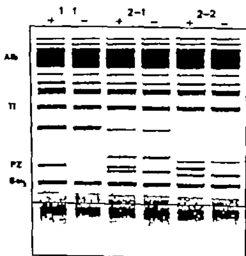


Fig 1 Drawing after original starch gels showing the electrophoretic mobility of the "pregnancy zone" (PZ) and the major serum proteins. *Alb* albumin; *Tf* transferring, *S* slow alpha, macroglobulin 1 1, 2 1 and 2 2 are the 3 haptoglobin types (+) and ( ) are the samples with and without the "pregnancy zone" respectively. The arrows show the directions of electrophoretic migration from the point of application.

Table I *Occurrence of the "pregnancy zone" protein in the sera of women taking oral contraceptives*

	"Pregnancy zone"		
	Absent	Present	Total
Contraceptives	72 (50)	73 (50*)	145
No co traceptives	72 (99)	1 (1*)	73

described in a previous publication (3) All sera were coded and tested blindly A schematic picture showing the electrophoretic mobility of the "pregnancy zone is shown in Fig. 1

From medical records information was obtained concerning parity type of contraceptive drug and time of administration.

The most common oral contraceptives in this investigation were: Conlunett (1 mg norethisterone and 0 1 mg mestranol), Follinyl (0.5 mg norgestrel and 0 05 mg ethinyloestradiol) and Anovlar mite (4 mg norethisterone acetate and 0 05 mg ethinyloestradiol). Other contraceptive drugs, like Lyndiol mite Ovulen, Protex and Delpregnin were used by rather few women and will be treated as a separate group in the presentation.

RESULTS

In the sera of 73 (50%) out of the 145 women taking oral contraceptive drugs the "pregnancy zone was demonstrable by means of starch gel electrophoresis (Table I) In the series of women not taking "pills" this zone was found in 1 patient out of 73 The difference with respect to the frequency of the "pregnancy zone protein between women taking oral contraceptives and those not taking oral contraceptives was highly significant ($P < 0\ 001$) There also was a significant difference in frequency of the "pregnancy zone" between different kinds of contraceptive pills (Table II) The largest difference was found between Conlunett and Follinyl ($P < 0\ 01$)

Table III shows the relationship between the

Table II *Occurrence of the "pregnancy zone" protein after administration of different contraceptive drugs*

	Pregnancy zone		
	Absent	Present	Total
Conlunett	21 (36)	37 (64)	58
Follinyl	28 (62)	17 (38)	45
Anovlar mite	13 (59)	9 (41)	22
Other	10 (50)	10 (50)	20

Table III *Relationship between duration of administration of oral contraceptives and occurrence of "pregnancy zone" protein*

	Pregnancy zone		
	Absent	Present	Total
<6 months	19 (45)	23 (55)	42
>6 months	53 (51)	50 (49)	103

duration of administration and the occurrence of the "pregnancy zone Administration of oral contraceptives for a short period (less than 6 months) resulted in about the same frequency of the "pregnancy zone" as administration for a period of more than 6 months.

Among women with no previous pregnancy the "pregnancy zone was observed in 61% compared to 45% for those with previous pregnancy (Table IV), this difference is not statistically significant ($0\ 1 > P > 0\ 05$)

DISCUSSION

The "pregnancy zone protein can be induced by contraceptive steroids and not exclusively by pregnancy The original observation by de Alvarez & Afonso (2) concerning the effect of Enovid is thus confirmed also for drugs with a different steroid composition. Among the women not taking oral contraceptives only one was found to have the "pregnancy zone protein. This woman had, however been delivered only 6 weeks before the examination and it is therefore possible that the occurrence of the pregnancy zone protein was due to the previous pregnancy The frequency of women producing the pregnancy zone is lower in this investigation than in that of de Alvarez & Afonso ( ) most likely due to the difference in steroid composition between the pills used Like Eno id Conlunett contains the

Table IV *Relationship between previous pregnancy and occurrence of pregnancy one protein*

	Pregnancy zone		
	Absent	Present	Total
N previous pregnancy	20 (39)	31 (61)	51
Previous pregnancy	52 (55)	42 (45)	94

oestrogenic component mestranol. The fact that Conlunett produced a significantly higher frequency of the "pregnancy zone" protein than other drugs raises the question whether the various oestrogenic components used in oral contraceptives differ with respect to their ability to induce the "pregnancy zone" protein.

The "pregnancy zone" protein may appear shortly after the onset of administration of contraceptive drugs. Thus de Alvarez & Afonso (2) found the "pregnancy zone" in 88% of women taking oral contraceptives during two cycles. The present results suggest that prolonged treatment (more than 6 months) does not cause a rise in the frequency of women showing the "pregnancy zone". The appearance of the "pregnancy zone" protein is therefore probably not due to an exhaustion of the metabolic "adjustment" mechanism.

The "pregnancy zone" protein, should probably better be named "steroid inducible α_2-globulin". In pregnancy it is obviously produced by the mother and not by the child which has been questioned in previous investigations (4). It is possible that the protein may function as a carrier or a defence mechanism for oestrogenic components. Experiments with the purified "pregnancy zone" protein are needed in order to obtain more information concerning its functional role in steroid metabolism.

ACKNOWLEDGEMENTS

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CONJOINED TWINS

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Abstract. During the years 1960 to 1970 inclusive, six conjoined twins were delivered out of a total of 403 441 deliveries including 3 276 twins and 37 triplet deliveries, an incidence of 1 in 67 241 deliveries and 1 in 655 twin deliveries. A seventh conjoined twin was delivered in late January 1971; inclusion of this case as well as the deliveries in January 1971 would lead to an incidence of 1 in 57 975 deliveries and 1 in 546 twin deliveries. Four of the twins were thoracopagus, one craniopagus, one dicephalus tripus tetrabrachius and one syncephalus. One of the thoracopagus twins occurred in a triplet pregnancy. All except two were delivered alive; four died within a few hours to a few days. One thoracopagus survived till 3 months of age; surgical separation was then attempted resulting in one survival who has developed normally and is now 3 years old. Antenatal diagnosis is necessary if the survival rate is to be improved.

Conjoined twins have fascinated both physician and layman over the centuries and many excellent papers have been written about them. In 1952, Sebastian Munster (12) reported on a case of craniopagus alive at the age of 6 years. About 400 to 500 cases of conjoined twins have been reported (3). Because of its extreme rarity many papers have been in the form of case reports and/or reviews of the subject. From available literature only one series of conjoined twins has been reported from a single hospital (11); this is because the condition is so rare that a single case may not occur for many years. As a result, the incidences reported are at best very approximate (8).

It is the purpose of this paper to present an analysis of the cases which occurred in the Kandang Kerbau Hospital, Singapore during the last 11 years.

MATERIAL

From 1960 to 1970, six cases of conjoined twins were encountered out of 403 441 deliveries giving an incidence of 1 in 67 241 deliveries. One of the twins was delivered before arrival at the hospital, but as the total deliveries in the hospital include all such cases (born before arrival), this does not affect the calculation of the incidence. This is compared with an incidence of 4 in 25 000 (11), 3 in 85 000 (10) and 2 in 100 000 (18). More recently Compton (2) reviewed five cases (from 2 hospitals) occurring in a period of 20 years, during which there were about 250 000 deliveries, an incidence of 1 in 50 000. During the same period, 3 276 twins and 37 triplets were delivered; the incidence of conjoined twins complicating twin gestations is therefore 1 in 655 and that for conjoined twins complicating triplet pregnancies is 1 in 37. Compton (1971) found an incidence of 1 conjoined twin in 900 twin deliveries but as the incidence of twin pregnancies for a single year was projected over 20 years to arrive at the total number of twins delivered during this period, the incidence must be considered to be only approximate. In the present paper, the total number of twins delivered were actually taken from the records. The incidence with regard to triplet pregnancies is most unreliable as the incidence of conjoined twins complicating triplet pregnancies is indeed very low. It is highly probable that no such case will be seen for many more years to come in the Kandang Kerbau Hospital.

A seventh case was encountered in January 1971; during this month there were 2 385 deliveries of which 12 were twin deliveries. Inclusion of this case together with the deliveries of January into this series would give an incidence of 1 in 57 975 deliveries (7 out of 405 826 deliveries) and 1 in 544 twin deliveries. Considering the extreme rarity of this condition, it is remarkable that the last three cases should have occurred within the last 6 months. The details of the seven cases are summarized in Tables I and II.

Age incidence

The maternal age ranged from 18 to 37 years with mean of 24 8 years which is very similar to the findings of other studies (Table III). Apparently maternal age has no influence on the likelihood of occurrence of conjoined twins. It has been thought that the commonest cause of conjoined twinning was the ageing of the ovum (19)- delayed ovulation with ov r-ripening of the ovum could be responsible for the gradual decline of the ability of the ovum to differentiate normally with the result that two centres of organisation, one unable t suppress the other would result.

Gravidity

This factor as in other studies (Table III) does not seem to influence the incidence of conjoined twinning either the gravidity ranging from 2 to 8 All the pregnancies except two had been normal with resultant normal deliveries. In the exceptions (cases 3 and 6) spontaneous abortions had occurred in preceding pregnancies before the case of conjoined twinning occurred. In case 1 the mother had five preceding normal infants before the conjoined twins resulted, subsequently she had two more normal pregnancies with normal babies before tubal ligation was performed. In none of the cases was there any history of twinning in the family The occurrence of twins (not conjoined) in preceding pregnancies has been reported by some authors (16, 20)- this would seem coincidental. I the genealogy of the famous Siamese twins, Chang and Eng Bunker more than 1 000 descendants hav been traced and only recently has a twin pregnancy been reported in any of them (1) It would seem generally that the most of the mothers have had normal pregnancies before delivering conjoined twin.

Pregnancy

Most of the pregnancies in the present series seem to have proceeded beyond 36 weeks. The duration of gestation is ery ariable in the many case reports that hav referred to it, some of the cases were abortuses while others reached full term (Table III). Three of the pregnancies in this series were complicated by hydramnios, in some cases ery severe; in case 1 there was no hydramnios, and case 4 was delivered before arrival, and since the mother had had no antenatal care no details of the pregnancy were available.

Hydramnios is present in a large proportion of such pregnancies (Table III) Hydramnios is a well known complication of fetal abnormality (14), and its presence in such a condition is therefore no surprise Its complication seemed to be more marked in the more severe cases as in case 6 where the condition was not compatible with life. In the review by Ripman (15), the incidence of hydramnios was present very frequently in those severe forms of conjoined twins (e.g. syncephalus or synpygus) that are apparently not compatible with survival.

Diagnosis

Clinically the diagnosis of multiple pregnancy can be made or suspected. It is only by radiology of the fetus that a diagnosis of conjoined twins can be suspected and in some cases made with cer tainty The diagnosis of thoracopagus can be made with some certainty if the heads are at the same level, there is an abnormal extension of the cervical spines with structural proximity of the spines, and if there is no change in the relative position of the structures after movement, manipulation and time (5) However these criteria do not apply to vertex presentations or those with only soft tissue fusion (15 17) In the present series the diagnosis was suspected in case 1 and made with certainty in case 6 (Fig. 1). In case 5 an X-ray was done at the 22nd week of gestation because multiple pregnancy was suspected, a diagnosis of triplets was then made. It was not possible t diagnose conjoined twins at this early stage of pregnancy especially in a case of triplets. The other four cases were only diagnosed in the second stage of labour when obstruction oc curred, two cases had no antenatal care, one of these two being delivered before arrival at the hospital. Thus except for case 6, diagnosis in all the cases was made during labour- this is not surprising as in most cases, conjoined twins are diagnosed during this stage of pregnancy (Table III).

Mod of delivery

Two of the babies (cases 4 and 6) were delivered *per vaginam*. Case 3 was delivered before arrival presumably by the breech as this was a case of thoracopagus tripus tetrabrachius, while case 6 was delivered by acuum extraction, this being possible because of the presence of only one head.

Table I *Details of the mother and the pregnancy*

Case	Year	Ethnic group	Maternal age	Gr	Previous obstetric history	Present pregnancy	Duration (Gestation)	Presentation at labour	Mode of delivery	Placenta
1	1961	Chinese	30	6	5 normal (singleton) pregnancies	Conjoined twins suspected from X-rays	38	Breech	E.U.A. when cervi almost fully dilated nd diagnosis confirmed. Electi caesarean section	Single (690 g) single cord
2	1964	Indian	19	2	1 normal (singleton) pregnancy	No nte-natal ca	—	Cephalic	Caesarean section for obstructed labour	Single (500 g); single cord
3	1966	Chinese	2.	5	1st and 3rd pregnancy borted	Twins, hy dramnios	41	Cephalic	Caesarean section for obstructed labour	Single 2 cords
4	1967	Chinese	—	–	—	—	—	? Breech	? Breech. Delivered before arrival t hospital	Single single cord
5	1970	Chinese	.3	2	1 normal (singleton) pregnancy	Triplets, hydramnios	38	Cephalic	Caesarean section for obstructed labour	Single (870 g) 2 cords, one for the single fetus, one for the twins
6	1970	Chinese	37	8	6 normal (singleton) pregnancies 7th borted	Conjoined twins diagnosed hydramnios	39	Cephalic	Vacuum extraction	Single (614 g) single cord
7	1971	Chinese	18	2	1 normal (singleton) pregnancy	Persistent breech	32	Breech	Caesarean section for obstructed labour	Single (484 g) single cord

E.U.A. = Examination under anaesthesia

Table II *Nature and outcome of the conjoined twins*

Case	Birth weight (g)	Sex	Type of conjoined twin	Outcome
1	3 860	F	Thoracopagus	Deli ered 1 Surgical separation done t ge 3 months ith one urv al
2	3 310	M	Thoracopagus	Fresh stillbirth (from asphyxia)
3	4 350	F	Craniopagus (ttachment at ertex)	Died shortly after delivery
4	3 860	M	Dicephalus tripus tetrabrachius (Sympygus)	Li ed for 4 day
5	4 765	F	Thoracophagus in a set of triplets	Died shortly after deli ery (from asphyxia)
6	3 060	F	Syncephalus (Cephalothoracopagus)	Gasped for few minutes
7	4 330	F	Thoracopagus	Fresh Stillbirth (from asphyxia)

…very				
…	Extraction	Destructive operation	Caesarean section	Outcome of infant
				2 lived
	11			1 lived
		6 →	1	All died
			5	All died
				15 stillbirths
4				2 lived
			5	2 lived
1				Lived
	0			
		2		Both died
			2	1 lived
				Died
	1			Died
		0		
			5	1 lived



Fig. 2. A thoracopagus.

Se

It is generally agreed that the majority of the conjoined twins are females, the ratio of female to male being 3 or 2 to 1 (3). Five of the seven cases were females again underlining the female preponderance in this condition. The dicephalus tetrabrachius had only one abnormal set of genitalia consisting of two 'scrotal' masses with no penis. However the cellular sex was male.

…pe of conjoined twin

…f the seven cases, four were thoracopagus, one …nalopagus, one syncephalus (cephalothoracopagus) and one dicephalus tripus tetrabrachius …ypygus). Thoracopagus is the commonest form …f symmetrical conjoined twins, in an analysis by …artuff of 117 cases, 86 w re thoracopagus, 32 …ygopagus, 7 ischiopagus and 2 craniopagus (16). …n all the cases of thoracopagus (Fig. 2) in this …eries, there were t o complete sets of organs, …ne for each member with the exception of … single conjoined liver much larger than normal (Fig. 3) … continuous diaphragm and … single pericardial cavity. In case 5 each heart had only two chambers, … entricle and an atrium—further details of this case will be published in a separate paper. In case 4 (Fig. 4) the upper parts of the babies were separate, the union occurring from midthorax and conti uing into a single pelvis with … single set of genitalia of doubtful identity, a single imperforate anus and three lower limbs (Fig. 5) one of which being apparently formed by a fusion of two limbs. There were two hearts in their separate pericardial cavities but one continuous diaphragm dividing the thoraces from the single large liver which was apparently the result of fusion of two livers at the midline (Fig. 4). The single stomach was posterior to the livers at the midline and continued into a single set of intestines terminating in an imperforate anus. The kidneys failed t … opacify during an intra… enous pyelogram and a retrograde cystogram demonstrated only a single bladder. Only tw… kidneys

Table III *Obstetrical aspects of conjoined twins*

Author	Nature of study	Age (y) Mean Range	Gravida Mean Range	Hydramnios	Time of diagnosis: Antenatal	Time of diagnosis: At labour	Time of diagnosis: At caesarean section	Gestation (weeks) Mean Range	Sex F M Ratio	Presentation Ceph B Ratio	Birth (g) Mean Range
Ripman (1958)	Review of 28 cases	26 1 19–35 (23)	2.6 1 9 (26)	13 (16)	4	23	1	35 28–43 (25)	14 11 (25)	18 9 (27)	3 650 1 136– 6 364 (22)
Milham (1966)	Review of 22 cases	23 20–34	2.4 1 5	?		?		33 19–40	20 1 ? "mixed" in 1 case		722 600– 5 280
Rudolph et al. (1967)	Personal series of 9 cases	28.3 18–39 (8)	2.6 1–4 (8)	?	3	5	0	38 6 30–42	9 0	2 5 combined" in 1 case (8)	4 777 1 650– 6 770
Compton (1971)	Personal series of 5 cases	1.8 16–35	3.2 1–10	1 (7 5)	1	3	1	38 9 36–41	4 1		4 445 870– 6 363
Tan et al. (1971)	Present series of 7 cases	4 8 18–37 (6)	4 1 –8 (6)	3 (6)		4	0	37.6 3 –41 (5)	5	4 3	3 934– 3 060 4765

Numbers in parentheses indicate number of cases with relevant information



Fig 1 A syncephalus in utero demonstrated by X-ray

The other cases were delivered by caesarean section but in only one case was this done electively" Rudolph et al. (17) were of the opinion that conjoined twins rarely caused dystocia because of their smaller size However in their review of the literature they found many instances of dystocia necessitating destructive operation or caesarean section they found that increasing numbers of conjoined twins were being delivered by caesarean section In a recent analysis ( ) dystocia was present in 3 of the 4 mothers who went into labour The present experience indicates that dystocia is a frequent and serious complication

Birth weight

The birth weight of the infants varied from 3 060 g in the syncephalus of 39 weeks gestation to 4 765 g in the thoracopagus complicating a triplet pregnancy (mean 3 934 g) The placentae in these cases were normal but of a size larger than usual In other reports the birthweight has ranged from a mere 600 g to over 6 000 g (Table III)

	…ery			
	Extraction	Destructive operation	Caesarian section	Outcome of infant
				2 lived
	11			1 lived
		6 →	1	All died
			5	All died
		7		15 stillbirths
4				2 lived
			5	2 lived
				Lived
	0			
		2		Both died
			2	1 lived
				Died
	1			Died
		0		
			5	1 lived



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Type of conjoined twin

Of the seven cases, four were thoracopagus, one craniopagus, one syncephalus (cephalothoracopagus) and one dicephalus tripus tetrabrachius (sympygus). Thoracopagus is the commonest form of symmetrical conjoined twins; in an analysis by Tartuff of 117 cases, 86 were thoracopagus, 32 pygopagus, 7 ischiopagus and 2 craniopagus (16). In all the cases of thoracopagus (Fig. 2) in this series, there were two complete sets of organs, one for each member with the exception of a single conjoined liver much larger than normal (Fig. 3) a continuous diaphragm and a single pericardial cavity. In case 5 each heart had only two chambers, a ventricle and an atrium—further details of this case will be published in a separate paper. In case 4 (Fig. 4) the upper parts of the babies were separate the union occurring from midthorax and continuing into a single pelvis with a single set of genitalia of doubtful identity, a single imperforate anus and three lower limbs (Fig. 5) one of which being apparently formed by a fusion of two limbs. There were two hearts in their separate pericardial cavities but one continuous diaphragm dividing the thoraces from the single large liver which was apparently the result of fusion of two livers at the [illegible] midline (Fig. 4). The single stomach was posterior to the livers at the midline and continued into a single set of intestines terminating in an imperforate anus. The kidneys failed to opacify during an intravenous pyelogram and a retrograde cystogram demonstrated only a single bladder. Only two kidneys



F g 3 Injection studies in thoracopagus demonstrating two hearts and one liver

were present and they appeared cystic. Injection of the vascular system post mortem demonstrated two aortic arches converging at the pelvis but not communicating (Fig. 6) In the craniopagus, the union was at the vertex (Fig. 7) the venous sinuses being continuous from one member to the other The pia and arachnoid separated the cerebrum of one twin from its fellow The syn

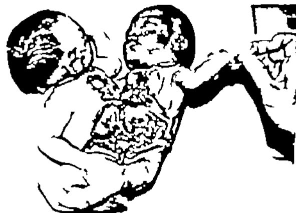


Fig 4 Dicephalus tripus tetrabrachius (case 4) ith t hearts, a large single liver and a set of abdominal viscera.



Fig 5 Dicephalus tripus tetrabrachius. Three lower limbs, one formed by a fushion of limbs The t o sacral dimples are prominent. Only one anus (imperforate) present.

cephalus will form the subject of another report there was only one heart and alimentary tract to the level of the diaphragm.

Fetal survivals

Five of the seven cases were born alive but four died shortly after The syncephalus though delivered without undue trauma died after gasping for a few minutes. Other cases of this nature have been reported (4 7) and they similarly failed to survive. It would seem that this type of mon-



Fig 6 Dicephalus tripus tet brachius I jection studies demonstrating tw aortae One bladde demonstrated on retrograde cystogram

strosity is too abnormal to be compatible with life. The dicephalus tripus tetrabrachius delivered before arrival at hospital lived for 4 days in spite of nonfunctional kidney and an imperforate anus. There was no means whereby this condition could be treated surgically: again it is obvious that this condition is too abnormal for survival. The craniopagus was delivered alive, but one member died shortly after and the other died while she was being anaesthetised in preparation for surgical separation. Though survivals have been reported with surgical separation of craniopagus twins (6, 20) it is evident that this is a very difficult operation needing great expertise and cooperation among the various hospital units. The present case was too ill for any planned operation to be done. All the thoracopagus twins could possibly have survived the delivery if the condition had been anticipated and elective caesarean section resorted to at or near term. However only in case 1 was the diagnosis made antenatally this case survived while the others died from asphyxia resulting from obstructed labour. In case 1 surgical separation was done at 3 months of age because of severe respiratory tract infection in one member. The operation resulted in one survivor who is now well with normal physical and mental development although torticollis and the defect in the anterior chest wall is still marked (Fig. 8) 9 years later. This case has been reported before (21).

Conjoined twins of the thoracopagus variety have not only a good chance of survival but also of normal development with modern surgical facilities and techniques. But because of the extreme rarity of this condition the diagnosis is often missed usually from failure to consider the possibility of this condition at pregnancy. It is therefore not surprising that the majority of them are born dead. However if greater awareness of this condition were present, it is probable that a greater number of survivals would result.



Fig. 7 Craniopagus. Fusion of the skulls was demonstrated.



Fig. 8 The only survivor, aged 9 years. The anterior chest wall defect is still marked.

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Fig. 3 Injection studies in a thoracopagus demonstrating tw hearts and one live

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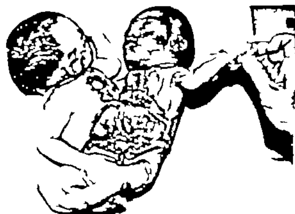


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Fig. 6 Dicephalus tripus tetrabrachiu I jection studies demonstrating two aortae. One bladder demonstrated on retrograde cystogram.

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INDUCTION OF ABORTION BY THE INTRAVENOUS ADMINISTRATION OF PROSTAGLANDIN $F_{2\alpha}$

A Critical Evaluation

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Abstract. Prostaglandin $F_{2\alpha}$ was administered intravenously to 166 women for induction of legal abortion. The patients were subdivided into different treatment groups to investigate the relative importance of the administered dose and the duration of infusion at various stages of pregnancy. The results of different parameters were analysed statistically. The details of the clinical outcome were analysed according to a defined scoring system describing the actual events following a certain treatment plan. Early pregnancies (7–8 weeks) were more susceptible to induction by $PGF_{2\alpha}$ infusion than later stages of gestation. The success rate in the 9th–20th week of pregnancy was dose dependent as demonstrated by termination of pregnancy in 75% of the high dose (100 μg per min) group and only 27% in the low dose group (50 μg per min). The duration of infusion was the second parameter of vital importance to achieve a satisfactory outcome. Infusion of a moderate dose (75 μg per min) for a period of 15 hours could induce valuable clinical response in the form of cervical dilatation, yet the duration was too short to complete the abortion process. The frequency of vomiting and diarrhoea was an important complicating feature in the high dose group. The high dose necessary for an acceptable success rate was accompanied by a high incidence of distressing symptoms (an episode of vomiting or diarrhoea every second hour). This fact limits the routine clinical use of intravenous $PGF_{2\alpha}$ prostaglandins for induction of abortion.

The biosynthesis of prostaglandins from polyunsaturated fatty acids, originally demonstrated in 1964 (3, 29), made sufficient quantities of these compounds available for clinical studies. The first report on the stimulatory effect of prostaglandin upon the pregnant human uterus in vivo was presented at the 2nd Nobel Symposium in 1966 (4). Following this preliminary study the uterine response to prostaglandin has been extensively investigated by several authors (5, 6, 11, 31). Shortly afterwards, reports appeared on the possibility of inducing labour by intravenous administration of prostaglandin (17, 18, 26). Clinical trials on the use of prostaglandins for induction of abortion were initially reported in January 1970 (19, 27). The possibility of using prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and E_2 (PGE_2) as abortifacients has subsequently been confirmed by many investigators (1, 7, 9, 12, 20, 21, 24, 33, 34). However, there has been a wide variation in the reported results concerning the proper dose, the efficiency of the method and the incidence of side effects. Accordingly this work was instituted in an effort to clarify these aspects and critically evaluate the potentials of this procedure.

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MATERIAL AND METHODS

Case material

This study was carried out on 166 women in the first and second trimesters of pregnancy. The patients were admitted to the hospital for legal abortion and volunteered for an induction trial with prostaglandin.

Prostaglandin administration

$PGF_{2\alpha}$ was administered by a continuous intravenous infusion. Electrical infusion pumps were utilized in approximately 50% of the cases while the remaining patients were given prostaglandin by a manually adjusted drip infusion.

The infusion rate was increased in a stepwise manner (25, 50, 75 etc. μg/min) at intervals of 1 1/2 hours until the desired dose level for each group was reached. Slight variations from the planned dose within each group

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Table IV *Induction of abortion by intravenous $PGF_{2\alpha}$ given over a period of 26 hours. Dose-efficiency relationship*

Duration of pregn (weeks)	Dose (μg/min)	No. of cases	Abortion No.		p^a
9–12	59	18	4	22	< 0 01
9–12	95	22	17	77	
> 13	46	12	4	33	< 0 05
> 13	99	39	29	74	

Calculated by χ^2 method.

infusion was almost doubled in the latter groups.

The accumulated data support the conclusion that the clinical outcome of prostaglandin induced abortion trials is related to the duration of pregnancy

Dose-efficiency relationship

The correlation between dose level and success rate was studied in a total number of 91 women. Thirty patients were given $PGF_{2\alpha}$ at a rate of about 50 μg per min and 61 women obtained approximately 100 μg per min. The infusion was maintained over a maximum period of 26 hours in both groups. Table IV shows that an acceptable success rate around 75 % was achieved in the high dose groups whereas the outcome in the low dose group was significantly lower (27 %). This difference was evident both in the 9th–12th week and the second trimester (≥ 13 weeks) cases.

It seems obvious that an infusion rate of 50 μg $PGF_{2\alpha}$ per min maintained for period of 26 hours is insufficient to achieve an acceptable success rate.

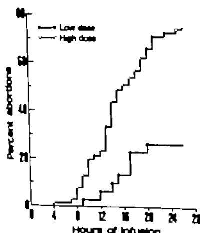


Fig 1 Cumulative percentage of abortions using intravenous infusion of $PGF_{2\alpha}$ for maximum period of 26 hours Duration of pregnancy 9–20 weeks. Low dose group (n 30) ≈ 50 μg min High dose group (n 61) ≈ 100 μg/min.

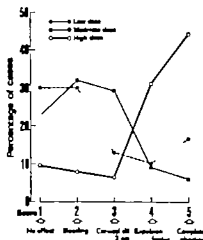


Fig 2 Clinical outcome following different administration schedules of intravenous $PGF_{2\alpha}$. The maximum period of infusion of the low (n = 30) and high (= 61) dose group was 26 hours, hile in the moderate (= 44) dose group the infusion was terminated following 15 hours. Note that 30 % of the patients in the moderate dose group had cervical dilatation of 2 cm or more.

Duration f infusion

The interval between the beginning of $PGF_{2\alpha}$ infusion and abortion varied considerably from one case to another This fact is illustrated in Fig. 1 which shows the increasing percentage of abortions during the course of 26 hours administration The data refer to pregnancies between the 9th and 20th week in the low and high dose groups. The curves seem to approach a sigmoid shape with the phase of maximum slope between the 10th and 20th hour Following this period, only few more cases are terminated if the infusion is continued. The mean infusion period to induce abortion was 14.3 and 15 0 hours in the high and low dose groups respectively ($p > 0.05$).

Detailed analysis f the clinical outcome

Abortion is preceded by bleeding as well as effacement and dilatation of the cervix. The degree of progress achieved at the end of the prostaglandin infusion is demonstrated in Table V and

Table I *Distribution of the patients into separate groups according to dose schedule and duration of pregnancy*

Group	No of cases	Dose (ng/min)	Infusion period (h)	Week of pregnancy
1	30	Low (50)	26	9–20
2	61	High (100)	26	9–20
3	44	Moderate (75)	15	9–20
4	31	Moderate (75)	8	7–8
Total	166			

were due to individual differences in tolerance and also due to difficulty in the precise adjustment of the drip infusion manually However the dose that each individual case had obtained was caref lly calculated in retrospect.

Supervision of the patient

A proforma specially designed for this study included complete history clinical examination and laboratory investigations. The latter included the blood picture, platelet count, erythrocyte sedimentation rate, haematocrit value serum creatinine, SGPT SGOT and urine analysis. The prof rma also included the following parameters. infusion rate blood pressure, pulse, side effects and vaginal examination findings, which were recorded at hourly intervals during the infusion period.

A staff of specially trained nurses supervised the patients continuously for dose adjustment and recording of side effects.

Selection of patient groups and dose sched le

The case material was subdivided into the following groups to illustrate the importance of the administered *dose and the duration of the infusion t diff rent stages* of pregnancy (Table I).

The first group received comparatively low dose aiming at a minimum incidence of side effects. In the second group, the dose was doubled in spite of the fact that a high frequency of side effects was expected. Unless abortion occurred, the infusions were maintained f maximum period of 26 hours in these t groups. The third group was designed to assess the efficiency of an infusion period of 15 hours, thus voiding supervision during the night. The fourth group served to evaluate the validity of previous results indicating that the early stages of pregnancy are more susceptible to the action of prostaglandin than the later stages.

Table II *Scoring system used at the end of the infusion period to evaluate the clinical outcome of the abortion trial*

Score	F nd ng
1	No effect
2	Bleeding only
3	Cervical d latation > 2 cm
4	Expulsion of the fetus, placenta retained
5	Complete bortion

Evaluatio of efficiency

Vaginal examination was performed repeatedly to record the occurrence of bleeding, state of the cervix and time of expulsion of the conceptus. To evaluate the degree of efficiency of the infusion, a pre-designed scoring system was followed (Table II).

Abortion was defined as complete or incomplete expulsion of the conceptus (score 4 or 5). In the early pregnant cases (7th–8th week), clinical examination cannot assure the occurrence of abortion. Immunological pregnancy tests on the urine before and after $PGF_{2\alpha}$ infusion, provided the evidence for the outcome of the induction trial in these cases.

The incomplete abortions were submitted to instrumental evacuation and failures were terminated by acuum curettage or hypertonic saline depending upon stage of pregnancy

Statistical analysis of the results

In the evaluation of efficiency the data were transformed and pproximated to normal distribution by using the formula given by Freeman & Tukey (14). The transformed data were then compared on the basis of χ^2-test. Student t test was utilized for the assessment of the remaining data.

RESULTS

Stage of pregnancy

Table III illustrates the efficiency of PGF_2 given as an intravenous infusion for a comparatively short period of time during different stages of pregnancy Using doses in the order of 65–75 μg per min there was a significantly higher success rate in the 7th–8th week than in the 9th–20th week of gestation ($p < 0.001$) Also there was a significant difference in this respect between the 9th–12th and ≥ 13th week of pregnancy ($p < 0.01$) The difference in success rate between the early and later stages of pregnancy is further emphasized by the fact that the duration of the

Table III *Induction of abortion by intravenous infusion of $PGF_{2\alpha}$. Relation to stage of pregnancy*

Duration of pregnancy (week)	M x. period of inf (h)	Dose (μg mi)	N of cases	Abortion No	
7–8	8	66	31	18	58
9–12	15	72	19	5	6
≥ 13	15	76	25	2	8

every patient. However this was less disturbing to the patients than the gastrointestinal symptoms. Vomiting and diarrhoea generally appeared in short episodes separated by symptom free periods.

Fig. 3 illustrates diagrammatically the incidence of vomiting and diarrhoea experienced by a random sample of patients in the low and high dose groups. It demonstrates clearly the frequent occurrence of episodes of vomiting and diarrhoea at a high dose level of $PGF_{2\alpha}$.

Table VI shows the incidence of vomiting and diarrhoea per hour of infusion in relation to different dose levels (9th–20th week of pregnancy). The occurrence of side effects was dose dependent as evidenced by statistical calculation. It is important to note that there was preponderance of diarrhoea to vomiting at low dose levels ($p < 0.001$). At higher dose levels this selective preponderance was not apparent ($p > 0.05$).

The relative incidence of vomiting and diarrhoea with the lapse of time was analysed in 28 women. An electrical pump infusion was used at a constant rate of 100 μg $PGF_{2\alpha}$ per min for 4 hours and the results were plotted graphically in Fig. 4. It was found that the percentage of volunteers experiencing vomiting or diarrhoea was approximately similar during the entire infusion period. This seems to indicate that there is no adaptation in this respect to the administered dose.

Hourly observation of blood pressure and pulse rate in all patients showed no change from the pre-infusion levels cept for one case who had transient drop in blood pressure for a few minutes. She recovered spontaneously without treatment.

Laboratory tests before the infusion, 3 days later and after 3 months, did not reveal any pathological changes, except for a few instances where leucocytosis was observed 3 days following the infusion. At the follow-up visit 3 months after the prostaglandin infusion it could be confirmed that all the patients had a return of regular menses after their abortion.

Table VI. *Incidence of vomiting and diarrhoea (episodes/hour of infusion) in relation to dose of* $PGF_{2\alpha}$

Dose (μg/min)	No. of cases	Vomiting Mean	Vomiting p	Diarrhoea Mean	Diarrhoea p^a
54	30	0.038		0.137	
74	44	0.125	0.01	0.111	0.05
97	61	0.228	0.01	0.242	0.001

Calculated by Student' t-test.

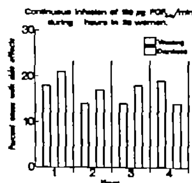


Fig. 4. Incidence of vomiting and diarrhoea in 28 women receiving $PGF_{2\alpha}$ intravenously at high rate. (This dose level was reached following period of stepwise increase in the infusion rate.)

DISCUSSION

The recent application of prostaglandins for the termination of pregnancy constitutes a new step towards the pharmacological induction of therapeutic abortion and possibly a method for post conceptive fertility control. The fact that an abortifacient agent is now available, demands a very careful evaluation to measure its relative merits and disadvantages.

Apparent discrepancies were observed between the results of different investigators with regard to the efficiency of intravenous $PGF_{2\alpha}$ for induction of abortion (7 9 19 21 24 34). This might be due to the differences in the applied dose schedules, the type of compound administered and the nature of the case material. Moreover the lack of uniform definition for "success rate" and incomplete data about the frequency and severity of side effects, has largely contributed to the present uncertain situation. The comparatively large number of patients involved in this study allowed a subdivision of the case material into different treatment groups. These groups could be compared with regard to the stage of pregnancy the doses used and the duration of infusion. The clinical outcome could be analysed not only in terms of success rate but also according to a defined scoring system describing the actual situation following certain treatment plan. The

Table V *Induction of abortion by intravenous $PGF_{2\alpha}$. Detailed analysis of clinical outcome*

Duration of pregn. (weeks)	Max. period of inf (h)	Dose (μg/min)	No of cases	Abortion: Complete abortion	Abortion: Placenta retained	Cervix dilated (> 2 cm)	Bleeding	No effect
9–12	15	72	19	2	3	6	7	1
> 13	15	76	25	1	1	7	7	9
9–12	6	59	18	2	2		8	4
> 13	26	46	12	3	1	2	1	5
9–12	26	95	22	10	7	1	2	2
> 13	26	99	39	17	12	3	3	4

Fig 2. More than half of the patients belonging to the low and moderate dose groups showed no effect or only vaginal bleeding as a result of the infusion. On the other hand 75% of the high dose group had a complete or incomplete abortion.

The degree of cervical dilatation reflects an important step in the chain of events leading to abortion. Thirty per cent of the moderate dose group cases presented cervical dilatation of 2 cm or more at the end of the 15 hours infusion This shows that although the moderate dose was sufficient to initiate the abortion process in a significant number of cases yet 15 hours of infusion were not enough to complete the abortion

Parity

In the low dose group there were 12 nulliparae and 18 multiparae with an abortion rate of 8 and 39% and an average induction–abortion interval of 16 6 and 14 8 hours respectively These differences were not statistically significant ($p > 0\ 05$) The corresponding figures for the high dose group were 28 nulliparae and 33 multiparae. The abortion rate was 68% and 82% and the induction–abortion interval 15 7 and 13 4 hours respectively Statistical analysis of these data did not reveal any significant differences ($p > 0\ 05$)

Side effects

Almost all patients involved in the study experienced side effects in one form or another and with different degrees of severity These included nausea, vomiting diarrhoea, epigastric or chest pain, cough, headache and blurring of the vision. Local erythema along the course of the vein utilized for infusion was observed in virtually

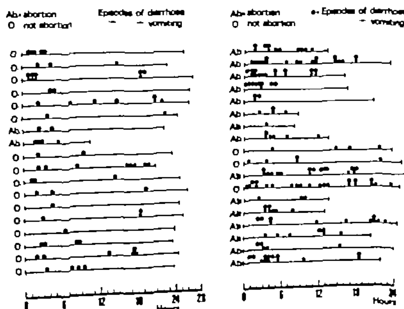


Fig 3 Episodes of vomiting and diarrhoea occurring in random sample of patients given intravenous infusion of $PGF_{2\alpha}$ Left low dose ≈ 50 μg min. Right high dose ≈ 100 μg min.

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Table VI *Incidence of vomiting and diarrhoea (episodes hour of infusion) in relation to dose of $PGF_{2\alpha}$*

Dose (µg min)	No of cases	Vomiting Mean	p*	Diarrhoea Mean	p*
14	30	0 038		0 137	
7	44	0 125	0 01	0 111	0 05
97	41	0 228	0 01	0.242	0 001

Calculated by Student' t-test

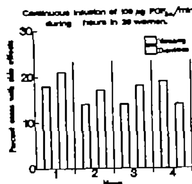


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evaluation was restricted to the authors participat ing in this study to achieve uniformity A specially trained staff of nurses supervised the administra tion of the proper dose schedule and provided means for a careful recording of the side ef fects.

The results of this study confirm previous reports of a significantly higher success rate in early pregnancies (7–8 weeks) in contrast to that achieved in later stages of gestation (7 33 34) Nyberg (25) had a similar experience of a higher rate of abortion in early pregnancies. This finding is further supported by the recently published results on vaginal administration of prostaglandin (22) where a high success rate could be achieved in very early pregnancies by using significantly lower doses than those necessary at later stages of gestation This difference in outcome between early and later stages of gestation is probably due to the reciprocal relationship between intrauterine pressures and the radius of the uterine sphere The same wall tension results in higher pressures if the radius is small, as it is in early pregnancies. This statement was also confirmed by recording uterine contractility (34) Furthermore the vascular structures surrounding the ovum in early pregnancy are more vulnerable to mechanical forces than is the fetus in mid-pregnancy sur rounded by protective liquor Thus uterine bleed ing appears early in cases before the 9th week of gestation (34)

The dose of PGF_2 is crucial in determining the effectiveness of the method. The doses of PGF_2 used by different investigators ranged between a minimum of 5 to a maximum of 200 μg per min However for a proper evaluation of the dose–efficiency relationship it is an obvious require ment that other variables (such as duration of in fusion and stage of pregnancy) should be com parable

Within an infusion period of 27 hours and a dose of 50 μg PGF_2 per min Karim & Filshie (19) induced 14 abortions out of 15 trials (93%) With approximately the same dose and the same duration, only 8 out of 30 (27%) pregnancies were interrupted in the present study Nyberg (25) administered PGF_2 at a mean rate of 83 μg per min (for a maximum infusion period of 24 hours) in 44 patients in the 9th–20th week of gestation Twenty-four pregnancies were terminated, i.e. a success rate of 55%. In another group in our study 61 patients received a dose close to 100 μg PGF_2 per min for a maximum period of 26 hours. In this group the success rate went up to 75% which is still lower than that presented by Karim & Filshie (19)

The reason for the marked difference in success rates between the results of Karim & Filshie (19) on one hand and that of Nyberg (25) and this study on the other hand is difficult to interpret. It might be argued that an overdose of a uterine stimulating substance results in reduction of the amplitude of the uterine contractions secondary to marked elevation of tonus. This mechanism may result in insufficient expulsive forces and uterine fatigue However studies of uterine contractility in this department did not indicate that a dose of 100μg PGF_2 per min would induce a sustained uterine contraction Moreover following an initial state of tetanic contractions, there was a decrease in the frequency along with an increase in the intensity of the contractions which reached a magnitude of 60–80 mmHg (28) Similar results were obtained by Hendricks who showed that the amplitude of the uterine contractions steadily in creased by a stepwise increment of the infusion rate up to 200 μg $PGF_{2\alpha}$ per min (15)

To the best of our knowledge, the present study is the only available one that includes two groups given low and high rates of PGF_2 infusion and conducted by the same investigators under iden tical conditions. It can therefore be concluded that the abortifacient efficiency of intravenous $PGF_{2\alpha}$ is dose dependent and that an acceptable success rate within a 26-hour infusion period can only be achieved with a dose of approximately 100 μg per min

The second parameter of importance for ac complishing an acceptable rate of success is the duration of PGF_2 infusion The following results may illustrate the influence of infusion period on the outcome

1 At a dose level of 75 μg per min and an in fusion period of 15 hours, there was a success rate of 16% (present study) Nyberg (25) ad ministered a dose of 83 μg per min for a period of 24 hours and achieved an abortion rate of 55%

2 At higher dose levels (100–110 μg per min) an infusion of 1 hours induced abortion only in one out of 6 cases (14) On the other hand with 4

hours infusion (present study) approximately the same dose resulted in 46 abortions out of 61 i.e. 75%

From these results it is obvious that an infusion period of 15 hours is insufficient even with high dose administration. It is, however believed that a prolonged $PGF_{2\alpha}$ infusion is more important in the second trimester of pregnancy than it is in the early stages.

The sigmoid curve shown in Fig. 1 indicates that only few additional cases abort after 20 hours of infusion. Furthermore the distress caused to the patient by an infusion period of 26 hours restricted, for ethical reasons, any prolongation of the treatment in our study

The a erage time needed to interrupt pregnancy in the high dose group did not differ in significant manner from that in the low dose group (14.3 and 15 0 hours respectively). This unexpected result may point to the possibility that, at certain dose level (high or low), there is a particularly susceptible percentage of cases that abort during the maximum slope period as shown in Fig. 1 Increasing the infusion period would have less dramatic effect on the remaining more resistant cases

Although the multiparae had higher success rate and a shorter induction–abortion interval than the nulliparae, both in the low and high dose groups, yet statistical analysis of these data revealed no significant correlation.

The detailed analysis of the clinical outcome by a scoring system demonstrated the effect of each plan of treatment and provided simple way of recording the different events at the end of the infusion. It can also provide basis for comparing the results of arious investigators. Utilizing this scoring system, an interesting feature was observed in the moderate dose group after 15 hours of administration about one third of this group had cervical dilatation of 2 cm or more. I 6-hour period, the high dose schedule can push the process of abortion towards completion, while a low dose ca only cause abortion in few susceptible pregnancies. The rest of the cases are unaffected or only show slight bleeding. Although, moderat dose can start the process of abortion, longer period of infusion becomes necessary for completion.

The incidence of side effects during the intravenous $PGF_{2\alpha}$ infusion and their relationship to the administered dose is acknowledged by almost all investigators in this field (7 9 19 21 24, 34). However in the individual case, the frequency of side effects such as vomiting and diarrhoea may vary during the course of the infusion from complete absence up to 25 episodes or more It seems therefore more accurate to measure these side effects rather than simply indicate the number of patients experiencing them.

In the high dose group the patients had, on the average one episode of vomiting or diarrhoea every second hour throughout the infusion period of 26 hours. This demonstrates the extent to which these side effects are an obstacle towards the practical and routine use of prostaglandin infusion for the termination of pregnancy However with low dose levels in the order of 50 μg per min, the side effects were not as frequent and could be reasonably tolerated by the patients. At such low dose $PGF_{2\alpha}$ seems to have a selective action on the myometrium and does not markedly exceed the threshold for stimulating gastrointestinal smooth muscle. With higher doses, such as those necessary to achieve an acceptable success rate, the threshold of stimulation of the gastrointestinal smooth muscle is definitely ex ceeded.

During the infusion there were no untoward effects on the patients' pulse or blood pressure with the exception of one patient who had a transient period of hypotension and a rapid spontaneous recovery this attack was probably related to subjective feeling of severe pain in a sensitive subject leading to a vasovagal response. Few patients complained of blurring of vision, which could be the result of a rise in intra-occular pressure (10, 30) Epigastric or chest pain, cough and bronchial irritation and facial flushing were oc casionally encountered symptoms and were often transient. The frequently observed erythema which extends for few inches along the subcutaneous eins used for the infusion caused little discomfort or alarm to the patients and subsided within couple of hours of stopping the infusion. I dermatological study Juhlin & Michaelsson (16) showed that the skin erythema seen after intradermal injection of PGF compounds was considerably smaller than that following the PGE compounds and w thout erythematous streaks or hyperalgesia.

There were no pathological changes in the laboratory tests performed 3 days and 3 months following the infusion except for occasional instances of leucocytosis. For interpretation of this finding a comparison with a control group of spontaneous abortions is needed.

Another question which remains to be answered is the possible teratogenic hazard following a failed trial to induce abortion by prostaglandin. In humans, this has never been investigated as other means are utilized to terminate pregnancy in therapeutic failures.

Our overall results of inducing abortion with intravenous $PGF_{2\alpha}$ are in accordance with the recent reports of other investigators (2, 9 24 25) At a comparable dose level the observed discrepancy between our results and those presented by Karim & Filshie (19 21) is difficult to explain. However the dissimilar case material, a difference in the definition of success and/or their administration of $PGF_{2\alpha}$ for a longer period of time may serve as possible explanations.

To keep the balance of effect on the target organ against their rapid clearance by the lungs, a continuous intravenous infusion of prostaglandin at a high dose level is necessary for a successful outcome This high dose infusion naturally precipitates a high incidence of side effects which in turn restricts the use of the appropriate dose.

Biochemical studies are needed to provide a prostaglandin compound with a slow clearance so as to allow a lower dose or few administrations to be effective It would be of great advantage if such a compound also had a better discrimination between its uterine and gastro-intestinal effects. PGE_2 seems to have a better selective influence than $PGF_{2\alpha}$ in this respect (9) However at the present time the only available means of reducing the side effects to a minimum is by local administration of the compounds into the uterus, either by extra-amniotic (13 32) or intra amniotic injection (8 23) The latter route of administration has proved to be highly successful and with a low incidence of side effects.

Following this attempt at a critical evaluation the relatively low success rate and the high incidence of side effects should lead to a reconsideration of the advantages of these compounds when administered intravenously and of whether they outweigh the other procedures currently in use for inducing abortion.

For the time being it seems logical enough to recommend termination of early pregnancies (≤ 12 weeks) by the simple operation of vacuum curettage since this procedure is still needed to evacuate the uterus when abortion is incomplete following a prostaglandin infusion. In the second trimester prostaglandins appear to have a definite role as their advantages and disadvantages compare favourably with the other procedures available at present. However the use of prostaglandins given into or around the amniotic sac seems to be superior and preferable to the intravenous route of administration in second trimester pregnancies.

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NORDISK FORENING FOR OBSTETRIK OG GYNÆKOLOGI
XVII KONGRES

Århus d 29 juni – 1 juli 1972

Hovedemne I *Koagulation og fibrinolyse i obstetrik og gynækologi*

Indledere Stormorken (Koagulationsprocessen) Inga Marie Nilsson (Det fibrinolytiske system) Lennart Andersson (Lokal fibrinolyse og fibrinolysehæmmere).

Koagulation og fibrinolyse hos ikke gravide (Rybo og Åstedt)
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Laboratoriediagnostik og kontrol (Stormorken og Hedner)
Antikoagulation (Storm)

Emnet behandles i form af et videre- og efteruddannelseskursus. Der vil blive adgang til diskussion, men ikke til anmeldelse af yderligere foredrag.

Hovedemne II *Obstetrisk analgesi og anesthesi*

Indledere J S Crawford, Birmingham (A General Survey of Obstetric Anesthesia) Michael Tunstall, Aberdeen (A General Survey of Obstetric Analgesia).

Paracervical block (Hans Westholm)
Hemineurin (Michael Tunstall)
Epidural analgesia (J S. Crawford)
An Evaluation of the Organisation of the British Anesthetic and Analgesic Service (Miss Josephine Barnes, London)
Panel Discussion
General Discussion

Et meget begrænset antal foredrag kan optages efter anmeldelse inden 1 april.

Frie foredrag: Anmeldelse til kongressekretæren inden 1 april 1972.

Kongressens adresse er: Kongressekretæren, Nørrebrogade 39 DK-8000 Århus C

Matti Grönroos	*Otto Resen Steenstrup*	*Mogens Ingerslev*
Generalsekretær	Kongressekretær	Præsident

Anmeldelsesblanketter og preliminært program udsendes til Nordisk Forenings medlemmer først i januar 1972. Andre interesserede kan rekvirere blanketter hos kongressekretæren.

Acta Obstet Gynec Scand 50: 391–395 1971

CASE REPORTS

PEPTIC ULCER IN PREGNANCY

Report of Two Cases of Surgically Treated Bleeding Duodenal Ulcer

Henning Becker Andersen and Vibeke Husfeldt

From the Department of Surgery (Head O Norring and H Pauby)
and Department of Gynaecology and Obstetrics (Head K. J Alling Møller), Central Hospital,
Nykøbing Falster Denmark

Abstract. 31 perforations and 28 cases of haemorrhage from proved peptic ulcer which occurred during pregnancy have been reported. The authors add 2 cases of bleeding duodenal ulcer near to term. These were treated in one case by Caesarean section plus partial gastrectomy at the same time and in the other case by partial gastrectomy during the puerperium. The oestrogen level and the greatly elevated histaminase concentration afford part of the explanation why symptoms, and especially complications, from peptic ulcer are so rare during pregnancy

Peptic ulcer and especially its complications, is rarely observed during pregnancy

The beneficial influence of pregnancy upon ulcer symptoms has been adequately confirmed (Chabannes, 1903 Sandweiss, 1951 Schmid, 1951).

Clark (1953), interviewing 400 women with proved peptic ulcer found that 88.2% had improved during pregnancy In 50% the symptoms had recurred 3 months post partum

The reported incidence of peptic ulcer during pregnancy is extremely varied Sandweiss et al (1943) found one case of peptic ulcer among 70 310 pregnant women, Johnston (1953) found cases among 12 041 pregnant women, and Durst & Klieger (1955) found 6 among 149 491 parturient women The true incidence is presumably higher as it may be difficult to diagnose the ulcer during pregnancy (Sandweiss et al., 1943 Clark, 1953 Baird, 1966)

Since le Play (1905) published the first case of ulcer complications in pregnancy Sandweiss et al reviewing the literature up to 1943 found 14 cases of confirmed complications. I Baird's review from 1966 this number had increased to 41 and we found another 18 (Table I).

Table II gives a survey of the types and treatment of the ulcers on the basis of Baird's (1966) findings and analysis of the cases listed in Table I

Since James (1948) reported the first successful operative treatment of a perforation, all detected perforations have been treated surgically without any maternal mortality whereas all perforations treated conservatively have had a fatal outcome.

Bleeding peptic ulcer has usually been treated by partial gastrectomy ever since Vasicka et al. (1957) described the first surgically treated case. If the operation is performed in the 3rd trimester the patient usually goes into labour in the course of a few days (Wendt et al., 1965 Dilts et al., 1967 Grosfeld, 1968). Two maternal deaths are on record, but both patients were in shock when the operation was started (Durst & Klieger 1955 Mobius, 1962).

Only 2 cases of Caesarean section and partial gastrectomy at the same time have been reported previously (Stevenson, 1962, Jones et al., 1969)

CASE REPORTS

Case 1 (CNF 3259 68–69)

A 26-year-old gr II with history of dyspepsia since the age of 15 and of pregnancy at 23 years of age The dyspepsia had subsided when she became pregnant, but returned immediately post partum and persisted until, at the age of 25 duodenal ulcer was diagnosed and treated by selective vagotomy and pyloroplasty Thereafter the patient was symptom free until the 26th week of the preg-

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out pregnancy, but she started to have pyrosis, eructations, pain in the epigastrium and below the right costal border, and later daily vomiting also.

In the 34th week (on 16.12.1968) the patient was admitted because of increasing pain. Her general condition was unaffected. There was mild tenderness below the right costal border and in the epigastric region. The uterus reached midway between the umbilicus and the xiphoid process. Hb 10.4 g/100 ml. The pain and vomiting grew more intense, and in the 35th week the patient had sudden fresh haematemesis, 1.5 litres in the course of 2 hours. By transfusion the blood pressure was kept above 120/80 mmHg, but the pulse rate was around 160/min. As the haematemesis persisted, it was felt that operation was indicated.

An asphyxiated girl weighing 2270 g was delivered by classic Caesarean section through a lower median incision. She scored 1 Apgar point, and spontaneous respiration was established after 5 min ventilation. Through the original incision a large ulcer was palpable in the pyloric region. Through another reversed V incision a solitary ulcer, 3 × 3 cm, was located in the posterior wall of the previous pyloroplasty. There was no fresh haemorrhage, but the ulcer was situated at the site of the gastroduodenal artery. There was no tumour in the pancreas. A Billroth II resection was performed, and the postoperative course was uneventful. Postoperative insulin test showed weak secretory acid secretion. Findings on X-ray examination of the sella turcica, opthalmoscopy and investigation of 17-ketosteroids were normal. The dyspepsia has not returned after the operation, and the baby has developed normally.

Case 2 (R.H.A. 131/68—R.H.C. 73/68–69)

A 20-year-old girl without any history of dyspepsia. The pregnancy had so far run a normal course and there was no anaemia. In the 37th week she began to have nausea, pyrosis, eructations, and discomfort. On the day before admission haematemesis occurred and there was fresh blood in the stools. This occurred again on the next day (15.4.1968), in the 38th week of pregnancy when the patient delivered a stillborn girl without assistance.

On admission the patient was pale and in pre-shock. BP 105/80 mmHg. Pulse rate 140/min. The abdomen was soft to palpation and non-tender. The uterus was contracted, 8 cm below the umbilicus. Hb 2.2 g/100 ml, haematocrit 10%. Two transfusions stabilized the condition. Two days after delivery the patient suddenly went into shock and passed a tarlike stool of 1 200 ml.

While receiving another transfusion she was transferred to Surgical Department C. After 10 transfusions the Hb rose from 2.0 to 12.8 g/100 ml. X-rays revealed a duodenal ulcer. Peak acid output, 39.0 mEq/h. Two weeks after delivery another haemorrhage occurred, the Hb dropped to 9 g/100 ml, and it was felt that emergency operation was indicated.

The operation disclosed arterial bleeding from a large callous ulcer on the posterior duodenal wall, penetrating into the pancreas. A Billroth II resection was carried out after ligation of the artery at the base of the ulcer. Such for technical reasons had to be left behind. In addition,

Table II *Summary of reported cases of perforation and haemorrhage from certified ulcers associated with pregnancy*

Present authors' two cases included

	Perforated ulcers	Bleeding ulcers	Total
Number of reported cases	31	30	61
Gastric ulcers	10	11	1
Duodenal ulcers	21	19	40
Results of treatment			
Non-operative treatment	17	16	33
Death, maternal	17	7	24
infant	11	7	18
Number receiving surgery	14	14	28
Type of surgery			
Caesarean section & simple closure	2	—	
Simple closure	11	—	
Vagotomy & pyloroplasty	—	2	
Partial gastrectomy	1	7	
Caesarean section & partial gastrectomy	—	3	
Caesarean section	—	2	
Death, maternal	—	2	2
infant	3	4	7

abdominal vagotomy was performed. The postoperative course was uncomplicated.

Autopsy of the infant: Stillborn, 3 150 g. Microscopic examination of the lungs: Severe aspiration of amniotic fluid, primary atelectasis.

DISCUSSION

The favourable influence of pregnancy upon peptic ulcer is well substantiated clinically and has also been demonstrated in animal experiments by Kelly & Roberts (1969) among others.

Oestrogens appear to be beneficial. Peptic ulcer occurs with the same frequency in both sexes before puberty whereas in the fertile age duodenal ulcer is 6 times more common in men than in women (Alsted, 1953). There is an unmistakable accumulation of new ulcers, exacerbation of symptoms, and complications around the menopause (Clark, 1953).

Clinical and experimental findings relating to the influence of progesterone and chorionic gonadotrophin have been conflicting (Bernstine & Friedman, 1948; Sandweiss, 1951).

The relationship between steroid therapy and peptic ulcer is well-known (Myhre, 1963). Gemzell (1953) demonstrated a steady increase in the corticoid concentration in the blood up to a maxi-

Table I *Summary of case reports not included in the references of Baird 1966 (when not otherwise indicated both mother and baby survived)*

Authors	Mother's age Pregnancy/ Parity	Ulcer complication Time	Type[a]	Treatment	Comment
Crisp, 1960[b]	36 multigrav	39th wk	H–G/D[*]U	Caesarean section	Shock treated by transfusions, amniotic fluid meconium coloured. Baby 4780 g, lethargic. Died 10 hrs later
	24 I/I	2nd day post partum	H–G/D U	Conservative	
	28 I/I	Term	H–DU	Conservative	Haematemesis 2 hrs post partum
Dilts et al., 1967	39 VI/III	7th mth	H–GU	Partial gastrectomy	Spontaneous delivery 2nd postop. day 1 750 g macerated stillborn male infant
Grosfeld 1968	19 I/I	36th wk	H–GU	V gotomy & pyloroplasty	"Gravid uterus made exposure difficult" 12 hrs later spontaneous delivery Postoperative wound infection.
Hennig et al., 1968	22 I/I	36th wk	P–GU	Caesarean section & simple closure	Perforation occurred after onset of labour Baby 2 600 g, pale asphyxiated. Autopsy massive aspiration of amniotic fluid
Honlotes et al., 1970	34 IV/II	4th–5th mth	P–GU	Simple closure	450 g stillborn infant delivered before admission. Severe abdominal pain 3 days before Perforation measured 10 3 cm
Jones et al., 1969	35 III/III	38th wk	H–GU	Caesarean section & partial gastrectomy	Amniotic fluid meconium coloured
	42 II/II	5th day post partum (term)	P–DU	Laparotomy-drainage of sealed perf	Abdominal pain from 4th day post partum. no muscle guarding" 7th day drainage of sealed duodenal perforation. 14th day drainage of lesser sac. Partial gastrectomy 4 months later
King et al., 1967	30 IV/IV	7th mth	H DU	V gotomy & pyloroplasty	Uncomplicated spontaneous delivery t term
McGarvey et al., 1952	41 III/II	2nd day post partum	P DU	Simple closure	no rebound tenderness or rigidity
Möbius, 1962	37 I/I	8th mth	H DU	Caesarea section	Caesarea section performed with pat. in circulatory collapse during blood transfusion. Pat. died. Baby died after tw respirations
	18 I/I	4th–5th mth	P–GU	Conservative	Pat died 17 days after sectio parva from peritonitis due t an undiagnosed perforation of gastric ulcer
	35 IV/IV	Sub partu (term)	P DU	Caesarea section & simple closure	Perforation occurred fter onset of labour
Tietz & Spellacy 1966	1 I/I	2nd–3rd mth	H–GU	Conservative	Ulcer verified by X-ray Uncomplicated delivery t term
Vasicka et al. 1957	40 VI/V	20th wk	H–DU	Partial gastrectomy	(At 33rd wk Caesarea section—hypertension and albuminuria) Baby died
Wendt et al., 1965	32 IV/IV	36th wk	H–GU	Partial gastrectomy	Only a 50 resection s done because of difficulties with the gravid uterus Following morning successful delivery by Vacuum extractor—2850 g
Winchester & Bancroft 1966	21 II/I	34th k	P DU	Simple closure	37th wk uncomplicated delivery

[a] H = haemorrhage GU = gastric ulcer P perforation DU duodenal ulcer
[b] Ulcers ecribed by X-ray Location not specified in first tw cases (Recorded as DU in T ble II)

ent pregnancy but she started to have pyrosis, eructations, pain in the epigastrium and below the right costal border, and later daily vomiting also.

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The relationship between steroid therapy and peptic ulcer is well-known (Mylius, 1963). Gemzell (1953) demonstrated a steady increase in the corticoid concentration in the blood up to a maxi-

mum of 7 times the normal level during the early days post partum. However Booth et al. (1961) have found this steroid excess to be protein-bound and consequently inactive.

Gastric secretion during pregnancy has been studied by Murray et al. (1957). The basal secretion was found to be reduced from the 10th to the 30th week. Histamine-stimulated secretion was reduced during the first 30 weeks, whereupon it rose steadily to normal levels around term.

Histaminase (review by Buffoni, 1966) which forms in the maternal portion of the placenta (Swanberg, 1950) is 400–1 000 times elevated from around the 7th week (Ahmark, 1944) This elevated histaminase concentration entails a significant reduction of the physiological response to histamine infusion (Törnqvist, 1968 *a, b*). The role of histamine in the mechanism of gastric secretion has not been definitely elucidated, but it has been demonstrated by Peter et al. (1963) that histaminase reduces the gastrin-induced gastric secretion in Heidenhain dogs. It also reduces insulin-induced secretion. Studies by Itoh et al. (1970) indicate that histamine takes part in the secretion mechanism as a chemical activator of the parietal cell.

CONCLUSION

The increased oestrogen level and the greatly elevated histaminase concentration appear to be factors in the reduced activity of peptic ulcer during pregnancy Complications are rare, and when they do occur it is usually in the 3rd trimester or immediately post partum. Perforation as well as major haemorrhage should be treated surgically on the same indications as in non-pregnant patients. If partial gastrectomy is to be carried out in the 3rd trimester it is advisable to start the procedure by Caesarean section. The foetus appears to be very sensitive to maternal circulatory failure which may occur in connection with the gastric surgery which is rendered even more difficult by the gravid uterus.

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Announcement

German Society for Endocrinology Competitions for 1973

Awards.	*Schoeller-Junkmann Award*	*Marius Tausk Career Development Award*
Amount	DM 15 000.–	DM 15 000.–
Donator	Schering AG Berlin	Organon G.m.b.H Munich
Applicants.	must reside in Europe and not be older than 40 years	must reside in Europe and not be older than 30 years
Subjects.	all fields of endocrinology (except diabetes mellitus)	clinical and clinical-experimental endocrinology (except diabetes mellitus)

Applicants are invited to submit two copies of previously unpublished papers, in either German or English, together with a short curriculum vitae and a description of the development of their scientific career to the President of the German Society for Endocrinology for 1972/1973 Prof H Schriefers, M D., 79 Ulm, Abteilung für Biochemie der Universität, Neubau Oberer Eselsberg, not later than October 15 1972

After receipt of the manuscript has been acknowledged by the German Society for Endocrinology the author is at liberty to have his paper published by a periodical.

Detailed information concerning the awards may be obtained from the President of the Society The awards will be presented at the 19th Symposium of the German Society for Endocrinology 1973

Acta Obstet Gynec Scand 50: 397–399 1971

PLACENTAL HAEMANGIOMATOSIS AND SEVERE MATERNAL ECLAMPSIA

Markku Seppälä and Eero Saksela

From Department II of Gynecology and Obstetrics (Head: Prof. P. Vara), University Central Hospital, Helsinki, Finland

Abstract. A case report is presented of an association of diffuse placental haemangiomatosis with severe maternal eclampsia. Eclampsia occurred at the 35th week of an otherwise uncomplicated pregnancy in a 20-year old previously healthy woman. An 1 800 g dysmature boy was born by caesarean section, and died in 12 hours from an apparent Klebsiella pneumonia. No organic malformations were found at autopsy. The placenta weighed 420 g and contained, on microscopic examination, characteristic diffuse placental haemangiomatosis without signs of infection or malignancy. The possible role of the placental lesion in relation to the acute eclamptic period is considered briefly.

The incidence of placental haemangiomata is high, as judged by a systematic examination of randomly selected sliced placentas, e.g. 6 in 600 (9), 7 in 500 (7), 4 in 562 (11), 8 in 620 (10), and 5 in 500 (2). Only a minority of the haemangiomata are visible on the surface of the placenta; the majority are situated within the placental substance and remain unnoticed unless the placenta is sliced (2, 10, 11).

Placental haemangiomata are usually circumscribed lobulated tumours consisting of three histological types (6): vascular, cellular and degenerate. A diffuse haemangiomatosis also exists where the borders of the process are indistinct and no clear-cut tumour is formed. The majority of haemangiomata are asymptomatic, but large tumours have been found to be associated with polyhydramnios or antepartum haemorrhage. Premature labour often occurs with these complications (3). A high perinatal mortality rate (31.3% (8) and 5.5% (1)) has been observed in association with placental haemangioma, and the occurrence of major fetal malformations is also high (about 10%) (1). We report a case where a previously normal 35-week-pregnancy was suddenly complicated by severe eclampsia associated with diffuse haemangiomatosis of the placenta.

CLINICAL HISTORY

The first pregnancy of a 20-year-old woman had continued until its 35th week without any complications. No proteinuria was present, and the blood pressure was not higher than 130/85 in any of the six routine checks before the attack. The patient's weight gain was 10.5 kg during the 34 weeks of pregnancy. Eclampsia occurred suddenly at the 35th week of pregnancy. The patient was deeply unconscious for four days. Proteinuria appeared, and her blood pressure was labile (from 80/– to 250/140). Aspiration, bilateral detachment of the chorioid, and anuria complicated her state. The electro-encephalogram showed marked pathological organic changes in the theta-delta region. The treatment of the patient included fluid infusions under central venous pressure control, tracheostomy, treatment in a respirator, hypotensive and anticonvulsant drugs (alcuronium, hydralazine, veratrine and diazepam). Caesarean section was performed at the onset of signs of fetal distress. A dysmature boy was born, weighing 1 800 g. The child scored 1 Apgar point at birth and died at the age of one day. No evidence of recent viral infections could be established, based on the titres of 23 antibodies. The autopsy of the child revealed a diffuse neonatal pneumonia, demonstrable both macroscopically and microscopically, and asphyxial petechial haemorrhages in various organs. No specific malformations were noted. In microbiological analysis a rich growth of Klebsiella pneumoniae was obtained from the lung specimens, and this infection was held as the immediate cause of death.

The recovery of the mother occurred slowly. At the re-examination two months later the ophthalmic changes had returned to normal, and the E.E.G. showed only minor changes.



Fig 1 A representative field from the placenta showing the diffuse angiomatous proliferation on the chorionic villi. Haematoxylin-eosin, ×250.

HISTOPATHOLOGICAL FINDINGS

The weight of the placenta was 420 g and its diameter was 15 cm. The outer surface of the placenta appeared normal. A routine biopsy specimen of about 2 × 2 × 2 cm was taken from the edge of the placenta. In the histopathological examination a diffuse change in the placental structure was observed in about two thirds of this specimen. Adequate information concerning the extent of the lesion in the placenta could not be obtained, since on macroscopical examination the placenta was deemed normal and destroyed. The histological features of the lesion are illustrated in Fig. 1

The chorionic villi were small and consisted practically entirely of capillary proliferation of vessels with flat regular endothelial lining. The lumina were filled with blood and no signs of mitotic activity or cellularity indicating malignancy were seen. The borders were indistinct and the portion of the placenta not involved in the capillary proliferation appeared normal and compatible with the gestational age The histological picture was characteristic of benign diffuse capillary haemangiomatosis of the placenta.

CONCLUDING REMARKS

There are reports indicating that placental haemangiomata could frequently be complicated by pre-eclamptic toxaemia (5). It has been suggested that toxaemia could be the result of either polyhydramnios or of proliferation of cytotrophoblastic cells in the tumour capsule (10) Neither of these was present in our case. Fox (3) found 10 toxaemias in 127 cases of placental haemangioma. This gives an incidence of 7.9 which is not significantly above that expected in any random series of pregnant women. The incidence of placental haemangiomatosis in toxaemia remains to be established. According to the incidence of toxaemia (4) the probability of an independent occurrence of the placental haemangiomatosis and toxaemia is about 0.0006 (0.01 × 0.06).

As the process in the present report was of the diffuse type which is a rare variant of placental haemangiomatous proliferation the occurrence of eclamptic toxaemia may be more than a coincidence Whether cases of this sort help in the understanding of the basic mechanisms underlying the toxaemic state remains to be seen. Various defects in the foeto-maternal barrier as well as in the foetal circulation could conceivably be caused by the haemangiomatous proliferation.

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World Health Organization Reports of Interest to Gynaecologists

No. 457/70. *The Prevention of Perinatal Mortality and Morbidity* 60 pp Price $1.25

This booklet is prepared by obstetricians, paediatricians and midwives. The representatives are: for gynaecology Professors A. C. Barnes, Baltimore D. G. Bonham, Auckland, M. K. Krishna-Menon, Madras, A. Ingelman-Sundberg, Stockholm and G. Tesauro, Naples; for paediatrics, Ass. Prof. F. Alison, Paris, Professors N. R. Butler Bristol, L. S. Prod'hom, Lausanne Ass. Prof. Ransome-Kuti, Lagos, for midwifery Miss D. J. Fairs, Norwich and Miss Huguette Merchiers, Brussels; and as the representative for Maternal and Child Health, professor Jamal K. Harfouche, Beirut. A survey is presented on the world situation concerning perinatal mortality and morbidity. Its causes are discussed and optimum care as well as care suitable for developing countries is proposed.

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These reference booklets give an excellent review of the actual progress within the different fields and are recommended.

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